Journal of Molecular Catalysis B: Enzymatic 107 (2014) 140–151

Contents lists available at ScienceDirect

Journal of Molecular Catalysis B: Enzymatic

journal homepage: www.elsevier.com/locate/molcatb

Review

Superactivity induced by micellar systems as the key for boosting the yield of enzymatic reactions

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ARTICLE INFO

Article history:
Received 11 November 2013
Received in revised form 3 June 2014
Accepted 4 June 2014
Available online 11 June 2014

Keywords:
Micellar enzymology
Enzymes
Surfactants
Ionic liquids

ABSTRACT

The superactivity phenomenon is a concept that expresses a significant increase on the enzymatic activity by common surfactants or ionic liquids emulsions. In this context, this work presents an overview of the literature on this subject, focused on the type, and characteristics of the surfactants and ILs reported in literature as superactivity inductors and the enzymes and reactions hitherto investigated in the superactivity context. It intends to emphasize the necessity of a multidisciplinary approach to this subject bringing together scientific communities of different fields to foster the understanding of this phenomenon, and to identify the type of reactions and processes that could and should be improved by it, having into account its potential application at industrial level.

Contents

1. Introduction

Many chemical reactions can occur spontaneously, others need to be catalyzed to proceed at a significant rate. Enzymes are proteins that perform essential roles to preserve the functionality and integrity of the biological systems through their catalytic function [1]. As any catalyst, enzymes act by reducing the energy barrier of the biochemical reactions. However, they display quite distinct properties when compared with chemical catalysts, being most of these properties a consequence of their complex molecular structure [1]. The high selectivity, the ability to operate under mild reaction conditions, high turnover number and high biodegradability are some of the enzymatic properties that make them potentially attractive for industrial applications [2]. The global market for industrial enzymes is estimated at $3.3 billion in 2010. This market is expected to reach $4.4 billion in 2015, a compound annual growth rate of 6% over the 5-year forecast period [3]. Major applications of enzymes are in medicine, textile industry, food and beverage industries and more recently, as analytical components [4]. However, enzymes are complex molecular structures that are intrinsically unstable and with high costs of production and purification, which are definite disadvantages with respect to chemical catalysts [5]. The overall impact of enzymes on industry is still below its full potential. In order to enhance the kinetics of enzymatic reaction, a large number of studies is focused in the application of different methodologies to increase or maintain the

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http://dx.doi.org/10.1016/j.molcatb.2014.06.001
1381-1177/

stability and activity of several enzymes, as discussed in recent reviews [2,6,7]. Some of these strategies include: (i) the isolation of intrinsically stable enzymes from extremophile microorganisms (capable of living in hostile environments considering the temperature condition), (ii) the genetic manipulation, (iii) chemical modification processes to stabilize unstable enzymes, (iv) their immobilization, (v) the inclusion of additives and finally (vi) the use of micellar systems and emulsions to promote the enhancement of catalytic reactions, here designated by micellar enzymology [2].

In the context of this review, the concept of aggregate systems and emulsions in the enzymology area will be highlighted. The catalytic potential of micellar systems has received special attention. In fact, in the living cell, enzymes exert their function in micro-heterogeneous environments interacting with, or being incorporated into, membranes [8]. Moreover, some enzymes can show a higher activity in presence of micellar systems or emulsions than in reference solutions (usually buffered solutions). This phenomenon is known as superactivity. Many investigations have been performed into the self-aggregation of common surfactants and their influence in the superactivity [9,10].

The purpose of this work is to present an overview of the type and/or more common characteristics of the surfactants reported in literature as superactivity inductors, *i.e.* the enzyme presents an increase of its catalytic activity when compared with buffered control systems, along with the enzymes studied and respective reactions investigated.

2. Surfactants: micelles, reverse micelles and microemulsions

The class of surfactants is defined as molecules with an amphiphilic nature, consisting in a hydrophilic head and a hydrophobic tail (normally composed of 1–4 chains) – Fig. 1.

In accordance with the nature of the head groups, the surfactants are normally classified into anionic (A), cationic (B and E), zwitterionic (C) and nonionic (D) (or amphoteric) chemical structures. These structures are able to spontaneously aggregate when in aqueous solutions to form micelles and, forming reverse micelles in organic solvents.

The micelles are described as aggregates originated by the auto-aggregation of surfactant molecules. In this case, the hydrophilic heads are oriented toward the dispersing solvent (generally water), while the hydrophobic tail(s) comprise(s) the interior region (normally designated as micellar core). As described by Langevin [11], the aggregates are the main form present above the critical micelle concentration (CMC), but free surfactant structures are also present in the system as monomer or small assemblies not organized in micelles.

The average number of surfactant molecules in a micelle, called the aggregation number, is dependent on the surfactant type and its concentration. In general, at low surfactant concentrations, micelles are composed by 100–200 surfactant molecules, being the aggregation number almost unaffected by the surfactant concentration. In other words, an increase in the surfactant concentration only leads to an increase in the micelles number [12]. Reverse micelles are aggregates of surfactant molecules where the head groups are oriented toward the polar core, being their hydrophobic tails into the non-polar medium. Reverse micelles, also called by some authors "micro-reactors" are characterized by the formation of water pools (located inside the micelles), where normally biomolecules with hydrophilic nature can be solubilized [13]. It is well-established that systems with enzymes acting in reverse micelles have two main advantages: (i) these systems can easily dissolve water-soluble, surface-active and water-insoluble substrates and (ii) these aggregates form a reverse micellar layer capable of to protect the entrapped enzymes which is acting against the deactivation of enzymes [14]. Reverse micelles are characterized by a definite diameter and molecular weight exhibiting a relatively ordered structure. Their diameters depend on the water-surfactant molar ratio described by the parameter W_0 [15], and increase with the water content [13].

$$W_0 = \frac{[H_2O]}{[\text{surfactant}]} \quad (1)$$

where $[H_2O]$ and [surfactant] are the molarity of water and surfactant, respectively. The major influence of this parameter is described considering two distinct scenarios; low amounts of water promote the formation of reverse micelles with a smaller size, while poor surfactant content leads to a lower number of microaggregates formed, which can be insufficient to maintain the catalytic activity of the enzyme presented in the biocatalytic system. Summing up, the amount of surfactant used in biocatalytic systems, when reverse micelles are applied is a crucial parameter to take into account [16].

The notion of microemulsion is applied to mixtures of at least three main components, namely oil, an aqueous phase, and the surfactant. Sometimes a fourth component, the co-surfactant, is added. Depending on the amount of each component present, the (micro)emulsion systems can vary between two extremes: from water in oil (w/o) (micro)emulsions, characterized by tiny water droplets dispersed in an oil phase, to oil droplets dispersed in a water phase (the so-called oil in water or o/w (micro)emulsion) [11].

The main difference between the micelles and microemulsions is the number of compounds used in their formulations. While micelles are a binary system, (micro)emulsions are ternary or higher order systems, being characterized by the presence of at least three components. The size of these aggregates can be different, being this parameter dependent on the surfactant type and concentration of dispersed phase. In some reports, the term w/o (micro)emulsions is used to refer reverse micelles [13]. The main characteristics of these three micellar structures are displayed in Fig. 2.

The micellar enzymology is devoted to the study of reactions catalyzed by enzymes in presence of surfactants, and it has attracted the interest of many researchers and industry [17,18]. The surfactants more commonly used in enzymatic studies are summarized in Table 1.

3. Enzymes and the superactivity phenomenon

Some enzymes are more active in micellar systems than in reference/buffered solutions [41,42]. This behavior is called superactivity and is defined as a significant increase in the activity of a specific enzyme due to the presence of micellar systems (as micelles, reverse micelles and (micro)emulsions) in the system bulk [13]. Resulting from the enzyme activity increase, the kinetics of reactions catalyzed by these enzymes are improved and the reaction yields increased. In order to understand this phenomenon and aiming at evaluating the new possibilities in the micellar enzymology field, several enzymes and reactions have been investigated. In this context, it is our aim to describe the results of diverse studies where the superactivity phenomenon is observed. Lipases and α-chymotrypsin (α-CT) are two of the enzymes for which the superactivity phenomenon has been more thoroughly studied in the catalysis of hydrolytic reactions. This section will thus be divided in three main sections: (i) serine protease α-CT; (ii) lipases and (iii) other enzymes.

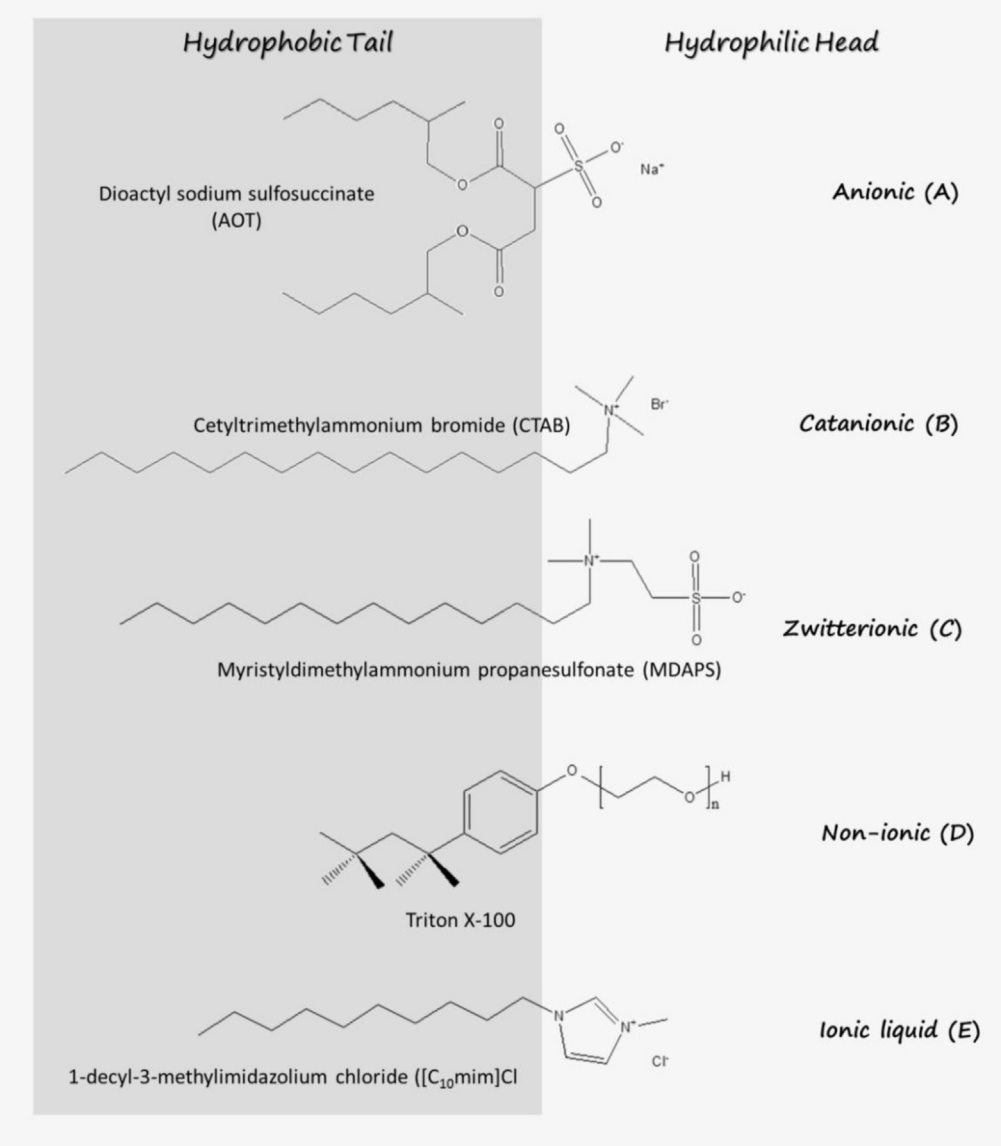

Fig. 1. Graphical representation of amphiphilic structures used as surfactants.

3.1. *Serine protease α-chymotrypsin (α-CT)*

The serine protease α-chymotrypsin (EC 3.4.21.1) is one of the most studied enzymes with a well-known structure and mechanism of action [43,44]. Many works in literature deal with the effect of surfactants on the α-CT activity and stability (some reviews are here highlighted due to the large number of articles dealing with this subject [45–47,6,48–53]). In those works, studies were carried out considering various surfactant types and concentration, and distinct water contents, being the kinetic studies emphasized and analyzed. However, in some of these works, it was sometimes observed a slight increase of the enzyme performance in presence of some surfactants [51,53], and thus the superactivity started to be considered. This prompted some authors to more carefully investigate this phenomenon associated with the α-CT and considering various substrates and distinct surfactants [24,28,54–60]. Alfani and co-workers [9,27,61,62] have carried out several studies using α-CT to catalyze the hydrolysis of *N*-glutaryl-L-phenylalanine *p*-nitroanilide (GPNA) in aqueous solutions of cetyltrialkylammonium bromide surfactants with different alkyl groups. In this work the main objective was to determine the effect of the size of the head group in the increase of the α-CT activity, being concluded that they are directly correlated (meaning that with the increase of the surfactant head, the superactivity phenomenon becomes more pronounced). These results were explained in terms of a positive correlation between the superactivity of α-CT and the surfactant hydrophobic nature [27,61]. More recently, Ghosh et al. [25] have studied the effects of the head groups of cationic surfactants on the hydrolysis of *p*-nitrophenylacetate (*p*-NPA). The results obtained are in close agreement with the aforementioned correlation. The authors also explained that, an increase in the head group size consists in the interfacial area increase, being the space between two head groups also enhanced [63]. This free space between two head groups allows the solubilization of α-CT at the interfacial region. Due to the enhanced interfacial region, both the enzyme concentration and the available substrate in contact with the enzyme increases, showing a higher activity. The dodecyltriphenylphosphonium bromide surfactant showed the best results in terms of the superactivity phenomenon, being the activity of the enzyme in the presence of the surfactant 9.4 times higher than the reference value (the activity of the enzyme in the buffer system without surfactant). Meanwhile, Spreti and co-workers [30] have investigated the effect

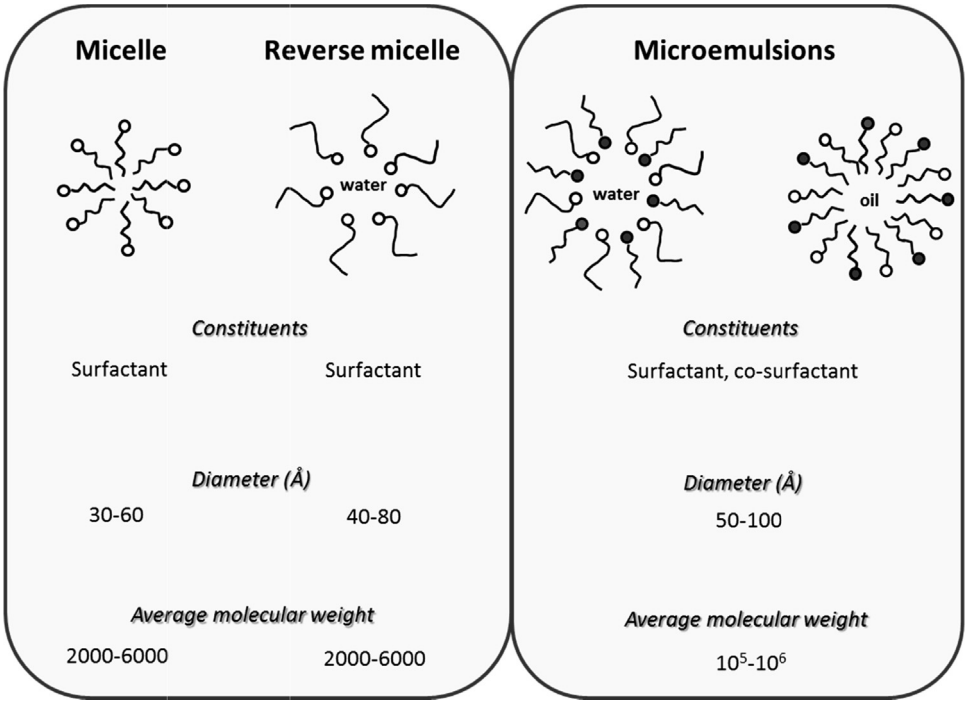

Fig. 2. Main characteristics observed for micellar systems.

of two distinct ammonium salts, the tetrabutylammonium bromide (TBAB) and cetyltributylammonium bromide (CTBAMB)with a bulky group on the α-CT activity toward three peptidyl subtracts. According to these authors [30], the addition of the two surfactants did not affect the enzyme–substrate affinities. Moreover, the authors pointed out that the α-CT activation was originated by the catalytically more favorable conformation of the enzyme induced by the presence of the additives. Furthermore, they sustain that the superactivity increase is strongly related with the alkyl chain length of the surfactant hydrophobic part. Thus, the dependence of the superactivity on the substrate is attributed by the authors to hydrophobic non-specific interactions between the surfactants and the enzyme active site, which produced a more hydrophobic microenvironment, and the consequent nucleophilicity increase of the catalytic active site. An alternative way of changing the surfactant hydrophobicity is by modifying the tail length, as described before and analyzed by different authors [28,48]. This is a change that let almost unchanged the characteristics of the micellar interface. Ghosh et al. [48] studied the kinetics of the hydrolysis of *p*-NPA in aqueous solution catalyzed by α-CT and adding a series of three surfactants alkyldimethylethanolammonium bromide (where the alkyl part being decyl, dodecyl and cetyl) at concentrations below and above their CMC. The results obtained suggest that the superactivity increases with the surfactant chain length, or in other words, with the increase of the surfactant hydrophobic nature. Several authors also report this correlation. Abuin and co-workers [28] used three alkyltrimethylammonium bromide surfactants (dodecyl, tetradecyl and cetyl) and the GPNA as subtract. The results obtained have shown superactivity in presence of these three surfactants, and maximum catalytic efficiencies closer the corresponding CMC. If the effect of surfactant is interpreted in terms of interactions between the enzyme and substrate, the increase of the chain length hydrophobicity would favor the formation of the self-aggregation of the surfactants rather than the interaction surfactant–substrate.

Moyano and co-workers [64] studied the hydrolysis of 2-naphthyl acetate by α-CT, through the application of cationic reverse micelles promoted by the presence of water/benzyl-n-hexadecyldimethylammonium chloride/benzene system. The catalytic performance of this cationic micellar system was also compared in the same study with the reverse micelles formed by the AOT surfactant (anionic system). The hydrolysis rates, the substrate partition constant between the organic and the micellar pseudophase, the catalytic rate constant and the Michaelis constant were analyzed, considering the effect of both reverse micelle systems. From these results, it was possible to conclude, for a constant W_0, that the Michaelis–Menten mechanism was valid for both micellar systems and that the reaction was taking place at the reverse micelle interfaces. It was also proved that the presence of the micellar systems was improving the catalytic behavior of the enzyme against its catalytic capacity in water. Furthermore, the authors mentioned that the α-CT efficiency is significantly higher for the cationic reverse micelles, when compared with the anionic system. The authors have explained this behavior by the enhanced hydrogen-bond capacity exhibited for the cationic micellar system due to strongly interactions between the enzyme and the cationic interface. The same reaction was used to test the effect of glycerol in reverse micelles of water/AOT/n-heptane and water/AOT/isooctane, considering the catalytic performance of α-CT [65,66]. The authors showed that glycerol was acting in the interior of the micelles and it is responsible for the higher thermostability and improved catalytic properties of this enzyme. In fact, the addition of glycerol as an additive promoted an increase in the efficiency of this enzymatic reaction of 8 times [65]. The superactivity considering the α-CT was also addressed in the work of Bru and Walde [67] by the measurement of the hydrolysis of *p*-nitroanilide and several esters. In fact, the superactivity was observed for the substrates becoming higher for low W_0 values and being described by the turnover number.

Celej et al. [57] suggest however that the higher α-CT catalytic efficiency in self-aggregates of CTAB results from significant enzyme conformational changes. In this work [57], it was observed an increase up to 80% in the yield of the *p*-NPA hydrolysis reaction

Table 1
Surfactants commonly applied in to enhance enzymatic reactions.

Surfactant	Type/action	References
Sodium dodecylsulfate (SDS)	Anionic	[19]
Sodium bis(2-ethyl-1-hexyl)sulfosuccinate (AOT)	Anionic	[20–24]
Dodecyltrimethylammonium bromide (DTAB)	Cationic	[19,25]
Dodecylpyridiniumbromide (DPB)	Cationic	[25]
Dodecyldimethylethanolammonium bromide (DDMEAB)	Cationic	[25]
Benzyldimethyldodecylammonium bromide (BDDAB)	Cationic	[25]
Dodecyldiethylethanolammonium bromide (DDEEAB)	Cationic	[25]
Dodecyltriphenylphosphonium bromide (DTPB)	Cationic	[25]
Cetyltrimethylammonium bromide (CTAB)	Cationic	[20,21,26–29]
Cetyltriethylammonium bromide (CTEAB)	Cationic	[26,27]
Cetyltripropylammonium bromide (CTPAB)	Cationic	[26,27]
Cetyltributylammonium bromide (CTBAMB)	Cationic	[27,30]
Cetyltriphenylphosphonium bromide (CTPB)	Cationic	[31]
Tetradecyltrimethylammonium bromide (TTAB)	Cationic	[28]
Hexadecyltrimethylammonium chloride (HTAC)	Cationic	[24]
Myristyldimethylammoniumpropanesulfonate (MDAPS)	Zwitterionic	[a]
3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS)	Zwitterionic	[32]
3-(Decyldimethylammonio)-propane-sulfonate (SB3-10)	Zwitterionic	[33]
3-(Hexadecyldimethylammonio)-propane-sulfonate (SB3-16)	Zwitterionic	[33]
Lecithin	Natural zwitterionic surfactant	[34]
Triton X-100	Nonionic	[22]
Polyethylene glycol monododecyl ether (Brij-30)	Nonionic	[21]
Polyethylene glycol dodecyl ether (Brij-35)	Nonionic	[35]
Polyethylene glycol hexadecyl ether (Brij-58)	Nonionic	[36]
Polyoxyethylene (2) oleyl ether (Brij-92)	Nonionic	[21]
Polyoxyethylene (20) sorbitanmonolaurate (Tween-20)	Nonionic	[21]
Polyoxyethylenesorbitan (20) monooleate (Tween-80)	Nonionic	[21,37]
N-gluconylglutamicacidddide-cylester (GGDE)	Nonionic	[38]
Octaoxyethylene dodecyl ether ($C_{12}E_8$)	Nonionic	[24]
nonaethyleneglycol mono-*n*-dodecyl ether – Lubrol PX (C12E9)	Nonionic	[32]
Ethylenediaminetetraacetic acid (EDTA)	Nonionic	[39]
Sorbitanlaurate (SPAN 20) and sorbitanmonooleate (SPAN 80)	Nonionic	[40]
Rhamnolipid	Biosurfactant	[37]

[a] Surfactant normally used, but not studied in the enzymatic superactivity until now.

in the presence of micelles when compared with the yield obtained in the absence of micelles.

Verma et al. [68] also observed superactivity for the hydrolysis of *p*-NPA, for the o/w microemulsions formed by CTAB as surfactant and n-butanol as the co-surfactant, respectively. The authors [68] have indicated the water content present in the microemulsions as the most important factor determining the reaction performance, being the higher superactivity observed for the lower W_0 values. This same group is attempting a more complete analysis and understanding beyond the use of reverse micelles and their effect on the α-CT performance. In addition to the results aforementioned, these authors believe that reverse micelles are attractive hosts for diverse enzymatic reactions since they are capable to solubilize both hydrophobic and hydrophilic parts of surfactants. Moreover, the authors mention that reverse micelles containing enzymes are acting like microreactors in which the enzymes can be protected from the media effects. This explains the crescent curiosity of the authors for this theme and thus, different works were developed and included in this field. In addition, Verma and co-workers used different surfactants, namely nonionic, zwitterionic and catanionic [31], to study the hydrolysis of α-CT considering also distinct substrates, the *p*-NPA [31,68] and the *para*-nitrophenyl benzoate (*p*-NPB) [31]. In 2010, the catalytic activity of α-CT in mixtures water/organic co-solvent (acetonitrile, dimethylsulfoxide, dimethyl formamide, ethylene glycol, methanol, ethanol, propan-2-ol, and *tert*-butanol) was studied considering the hydrolysis of *p*-NPA and *p*-NPB [31], by applying the CTPB surfactant. This work showed the influence, not only of several organic solvents, but also different temperatures and the presence of a cationic surfactant, the CTPB, associated with the hydrolysis reaction here discussed. The kinetic parameters were observed and from their analysis, it was possible to identify the optimum temperature of 27 °C. The surfactant was also tested in terms of its effect on the hydrolysis reaction, and it was clearly shown that the presence of the CTPB was improving the enzyme activity, which was justified by the increased flexibility of the enzyme when in presence of the CTPB surfactant [31]. Recently, in 2013 [33], the same group investigated the effect of reverse micelles composed of three distinct surfactants, the nonionic AOT, and the zwitterionic detergents 3-(decyldimethylammonium)-propane-sulfonate (SB3-10) and3-(hexadecyldimethylammonium)-propane-sulfonate (SB3-16). In this work, the effect of the surfactants was studied in terms of activity, stability and kinetic parameters, having into account α-CT. In this work, the influence of mixed surfactant systems was assessed. The authors highlighted as main conclusions of this work that, the size of the water pool formed is responsible for significant changes in the enzyme performance. Moreover, they demonstrated that, in this particular case, the use of mixed reverse micelles [AOT/isooctane/mixed surfactants (SB3-10, SB3-16 and Triton X-100)] is improving more the enzyme activity, when compared with the simpler reverse micelle formed by AOT/isooctane, at fixed conditions of temperature and pH [33].

In summary, the available literature provides indications and guidelines about the importance of the surfactant hydrophobicity (in what concerns both the increase in the head group size but also of the tail length), and the water content present in the micellar bulk to the superactivity phenomenon when the α-CT activity is considered. Changes in the enzyme conformation have been suggested but more studies are necessary to clarify this issue. A comprehensive understanding on the superactivity molecular origin remains yet elusive, in particular the interactions between the surfactant and the enzyme. A more complete study with different types of surfactants is mandatory for an understanding of the effect of the surface charge of the microemulsions and an optimization of the superactivity phenomenon aiming at its application to reactions of industrial interest.

3.2. Lipase

Lipases (EC 3.1.1.3) are water-soluble enzymes that play a key role in fat metabolism and digestion by cleaving long-chain triglycerides into polar lipids [69]. This group of enzymes is a powerful tool for catalyzing not only hydrolysis, but also esterification and transesterification reactions involving water-insoluble esters [70]. It has long been recognized that lipases have only a marginal activity toward dissolved substrates in aqueous solutions but show high activity when the substrate concentration is high enough to form self-aggregation (that is, in presence of an aqueous–organic interface) [69]. In fact, the majority of the reactions catalyzed by lipases are performed in the water–organic interface, being the "interfacial activation" involved in the catalytic mechanism. As explained by Guncheva and Zhiryakova [10], in an homogenous bulk, the lipases are in an inactivated state, since the lid that is isolating the active site from the polar solvents is characteristically non-polar. When a second organic or lipid phase is added, the lipase is located at the interface and the lid undergoes conformational displacement, allowing the availability of the interior hydrophobic part of the enzyme to the substrate, which is described by the "interfacial activation" stage. Hence, interfaces can be considered the key spots for lipase biocatalysis [70], not only because the enzyme affinity to work at the interfaces, but also because in the particular case of reverse micelles, this interface is protecting the enzyme from the organic bulk [71]. Not surprisingly, the presence of a surfactant in the lipase catalysis has become of major interest as a vehicle to enhance the enzymatic activity. This is justified by the increase in the water–lipid interfacial area that, in some cases and for some reactions, is helping in the "interfacial activation" stage. Moreover, the surfactants are sometimes also responsible for the increase of the substrate solubility, stabilizing the open conformation of the enzyme and preventing it from aggregation, which contribute for a better catalytic performance of the lipases [10]. Indeed, much higher enzyme activity in reverse micellar systems than in bulk water has been reported for some enzyme–substrate systems [53], because most of the substrates are water-insoluble.

Malakhova and co-workers [13,72] are pioneers in describing the lipase hydrolytic activity in microemulsions. In this work, the hydrolysis of triolein and tributyrin by a pancreatic lipase in an AOT/octane microemulsion system was assessed. Since then, these (micro)emulsions systems, based on synthetic or natural surfactants have been used for the lipase-catalyzed hydrolysis of vegetable oils and triglycerides [13].

The anionic surfactant AOT has been widely used in the field of micellar enzymology in which surface active enzymes like lipase show superactivity [73–76]. Miyake et al. [76] observed superactivity using a lipase from *Rhizopus delemar* was, to catalyze the hydrolysis of 2-naphthyl acetate (a hydrophobic substrate), both in aqueous solutions and in w/o (micro)emulsions formed by the surfactant AOT and heptane as co-surfactant. Yamada and co-authors [73] have used the AOT to study the modification of AOT/isooctane reverse micelles with various alkyl glucosides (AG_8, AG_{12} and AG_{18}) and nonionic surfactants on the *Chromobacterium viscosum* lipase. The authors were capable of modifying the AOT reverse micellar systems, with the addition of various alkyl glucosides and the nonionic surfactants, aiming at improving the activity of the lipase. They explain that different micelles are obtained since for example the Span-surfactants were solubilized into the micellar water pool, while the Tween-, Triton-surfactants, and the alkyl glucosides were solubilized at the interface of the AOT micelles, forming mixed micelles. The authors were also describing the hydrophobicity of the systems as an important condition since, by the addition of nonionic surfactants to AOT reverse micellar systems, particularly Tweens and Tritons conjugated with poly-(oxyethylene) chains, in the improvement of the lipase activity [73]. The authors also mentioned that the location of the substrate is crucial, because this is related to its accessibility. Since different substrates were investigated in this work, it was proved that the distribution of substrates into the micellar water pool, micellar interface, and bulk organic phase has an important role in the activity of the lipase. More recently, Shome and co-workers [21] have been successful in using cationic w/o microemulsions as the key to enhance the lipase activity through the addition of a nonionic surfactant. The authors [21] evaluated the effect of several w/o (micro)emulsions, prepared from CTAB and four different nonionic surfactants (Brij-30, Brij-92, Tween-20, and Tween-80). The reaction was performed using the *C. viscosum* lipase (*Cv*-lipase). The results show that the lipase activity was enhanced up to 200%, and that this significant increase is related with the increase in the content of nonionic surfactants present in the microemulsion systems, when compared to CTAB alone, despite the good performance of the latest. This behavior is justified by the interactions formed between the anionic and cationic surfactants that are responsible for the reduction of the positive charge density at the microemulsion surface. According to these authors, the enzyme activity is improved simply by the reduction of the capacity of the cationic surfactant to inhibit the enzyme active site [21]. Furthermore, the authors explain that the nonionic surfactants, such as the Brij, provide an increased flexibility to the interface, contributing for the stability of the microemulsions formed. Conflicting results are reported by Polizelli et al. [29] that investigated the effect of two anionic (SDS and SOS), three cationic (DTAB, TTAB and CTAB) and two nonionic (Triton X-100, Tween-80) surfactants on the activity and stability of a lipase extracted of oilseeds from *Pachira aquatic*, using the *p*-NPA as the subtract. Moreover, three different polymers (polyethylene glycol – PEG 0.2, 8 and 12 kDa) were studied in a large concentration range. This work reports that anionic and nonionic surfactants showed inhibitory effects in the complete range of concentrations studied. The authors report that probably, interactions between the substrate and the surfactants exist blocking the active center [29]. However, all the cationic surfactants were capable of promoting an increase in the lipase activity. CTAB for example, showed a clear stimulatory effect, inducing an increase in lipase activity higher than 32%. This effect seems to be proportional to the surfactant alkyl chain length (CTAB > TTAB > DTAB). It seems that this behavior can be also justified by micelle formation, because the CMC of CTAB is lower, facilitating the substrate–enzyme interaction [29]. Meanwhile, the authors also report a positive effect of the presence of all the polymers and in the whole range of concentrations studied. In fact, the hydrolytic activity in aqueous solution as increased from a maximum of 200% for the PEG 0.2 kDa until around 500% for the PEG 12 kDa. The work reports that the increase in the activity is proportional to PEG molecular weight, which can be justified by the enhanced hydrophobic nature promoted by the increase in the molecular weight of the polymers and then, facilitating the access of the substrate to the active site [29]. Another explanation advanced by the authors is related with the reduced water activity for the high polymer weight, favoring the exposure of the hydrophobic residues in the opening lid of the PEG, or the enzyme aggregation [29].

The impact of the introduction of hydroxyl groups on the surfactant head-group on the activity of *Cv*-lipase, considering the hydrolysis of *p*-nitrophenyl caproate in w/o microemulsions was reported by Das et al. [20,77]. The impact of the surfactant head-group size on the activity of *Cv*-lipase [20] was firstly studied. For that purpose, different surfactants (Fig. 3) were prepared by replacement of the three methyl groups of CTAB with hydroxyethyl (series I), methoxyethyl (series II) and n-propyl groups (series III) [20].

Notably, the activity remained almost comparable for the analogs of both series I and III, in spite of the drastic change in the hydrophilic nature of the surfactants. The lipase activity clearly

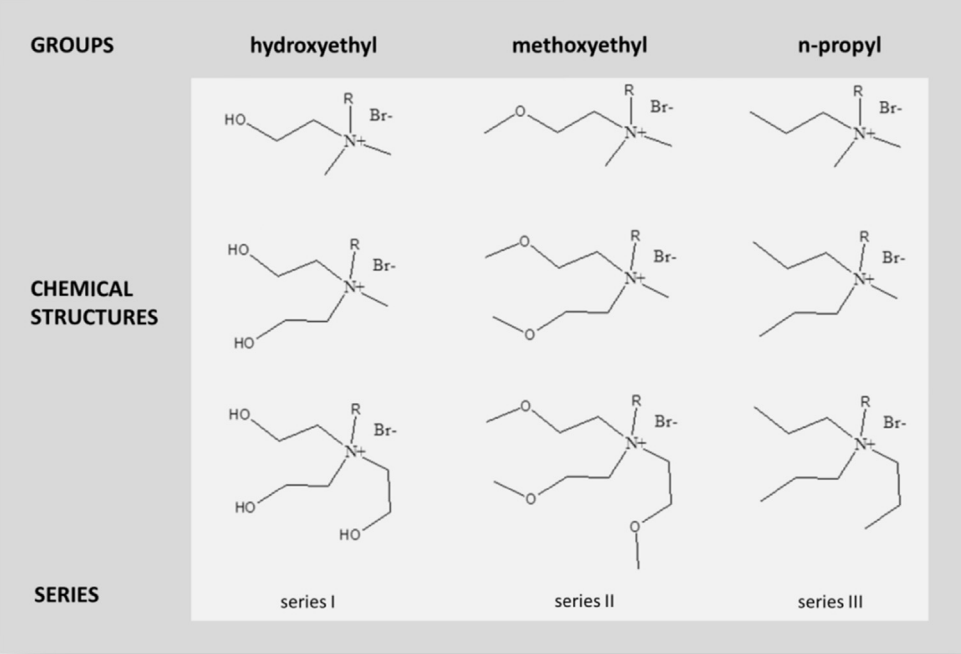

Fig. 3. Chemical structure of the three different series of surfactants investigated in the Das et al. [20] work.

increases with the head-group size regardless of its hydrophilic nature [20]. Thus, the activity was observed to increase when the series II is applied, and comparing with the most hydrophilic surfactant structures (series I). Meanwhile, the activity of the lipase is identical when series I and III are compared, independently of the significant differences in their hydrophilic nature. Meanwhile, the authors [77] established that the introduction of hydroxyethyl groups at the polar heads of the surfactants dramatically enhances the catalytic efficiencies of lipase by increasing the interfacial content of water. In fact, the hydroxyethyl groups not only increase the hydrophilicity of the surfactant, but at the same time, the size of the head group and consequently, the surface area *per* head group at the interface. Moreover, the authors explain that the lipase catalytic efficiencies are dramatically enhanced due to the increase of the water content in the interface originated by the formation of w/o microemulsions. This report is showing that an increase in the enzyme activity up to 4- and 10-fold, from 1 to 4 hydroxyl groups attached to the head group of the surfactant, respectively, when compared with the use of cationic w/o microemulsions. The conclusion was that the head-group size was responsible for the regulation/increase in the enzyme activity. This is explained by the correlation between the head-group size and the higher space available for the enzyme to attain a more flexible conformation, as well as facilitating the increase of the local concentration of enzyme and substrate, leading to the higher efficiencies of the lipase (Fig. 4).

As mentioned above, the interfacial area is other parameter that influences the lipase activity in an emulsion system. As previously discussed, enzymes located at an augmented domain in reverse micelles always exhibit a superior activity due to the increased substrate concentration and conformation flexibility of the biocatalyst. A recent work [26] using thiol (1-dodecanethiol and 1,6-hexanedithiol)-assisted confinement of gold nanoparticles (GNPs) at the interface of reverse micelles composed of three different surfactants was reported, aiming at increasing the interfacial area. In this work, the authors attempt at understanding the influence of this latest parameter, meaning the interface of the micelles, in the enzyme activity using the *Cv*-lipase. The thiol-assisted confinement of appropriately size of the GNPs at the reverse micellar interface was proved to be an advantageous strategy to increase

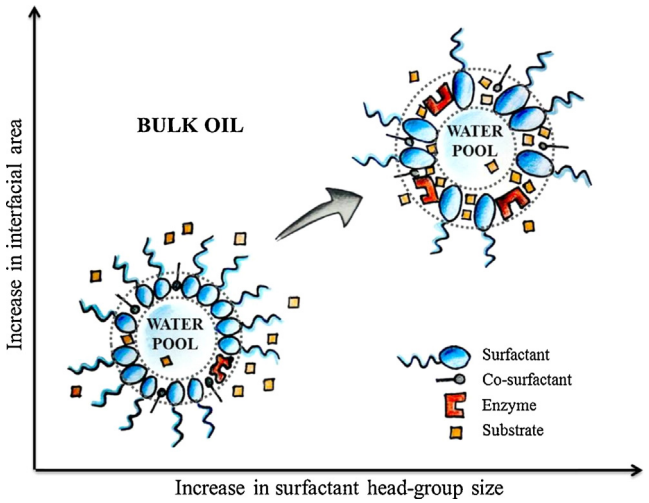

Fig. 4. Schematic illustration of the main conclusions obtained in the Das et al. [20] work, which is representing the effect of the head group size in the micelle structures.

the *Cv*-lipase activity, due to an appropriate enhancement in the overall interfacial area of w/o microemulsions.

As recently reviewed, the lipases from *Bacillus* are tolerant to the presence of small amounts of triton X-100 but are only superactivated in presence of higher amounts of this surfactant [32,78]. The authors also report that the presence of other surfactants is positively affecting the superactivity of the thermoalkalophilic lipase of *Bacillus thermocatenulatus.* In fact, different surfactants such as the Lubrol PX (0.1%), Tween 20, CHAPS (1%, w/v) and Brij-35 demonstrated their ability to increase the lipase activity in, respectively 139%, 137%, 184%, and 163% [32]. However, in this work, a careful discussion about the effect of different structural alterations of the surfactants was neglected.

Ruiz and co-workers [79] have studied the effect of different surfactants, namely SDS, EDTA, PHMB and fatty acids (capric acid, and myristic acid) in the catalytic behavior of lipases from *Bacillus* in the hydrolysis of *p*-nitrophenyllaurate (*p*-NFL). In this work, different

concentrations of the fatty acids were applied. The main conclusion obtained was the increase in the enzymes activity induced by the surfactants and fatty acids used. In this sense, the enzymes were activated 110.1%, 120.5%, 156%, 231% to 292%, considering EDTA, PHMB, capric acid, lauric acid, and myristic acid, respectively. Finally, the authors have concluded that, different concentrations of fatty acids may have different effects on the enzymatic activity [79]. Despite the large number of articles dealing with lipases and surfactants, very few address the superactivation of the enzyme in presence of various surfactants [10,34,37,80]. Having this in mind, and the main results and conclusions described in the present work, it seems that some systematic studies should be performed with a variety of surfactants to achieve a better understanding about the interaction with the various surfactants on the superactivation of the enzymes.

Summing up, the authors have until now been dealing with various surfactants, where the concentration and hydrophobic/hydrophilic nature, described by the size of the head group were assessed parameters, being however (sometimes) difficult to understand the real effect and interactions that control the superactivity phenomenon for lipases. Despite the larger number of works dealing with lipases, inconsistencies between different authors are paten. These discrepancies are normally defined by comparisons between different micellar systems, for example reverse micelles and mixed micelles [21,29]. Moreover, the authors claim that different lipases, reactions, substrates [73] or even different surfactant concentrations have a responsibility in these discrepancies, since the distribution of the substrates, surfactants, the structure of the interfacial layer, and the location of the enzyme are different [81]. Meanwhile, some results seem to establish the effect of the water activity as an important parameter to take into account. In general, for reverse micelles it is well established that the water present in the systems has different properties and is dependent of the location of the water molecules, since the water located inside the reverse micelles and in close contact with the surfactants differs from those molecules concentrated in the bulk or central part of the reverse micelle. The authors call thus the attention for the differences in the physical properties between the two water layers. Moreover, the superactivity phenomenon remains inconclusive in terms of the interactions 'surfactant + lipase', 'lipase + water', 'water + surfactant' and 'enzyme + surfactant + water'. Deeper and more comprehensive studies are required using different surfactant types, focusing in reactions with industrial importance (since lipases are one of the classes with higher industrial applicability [47]), different amounts of water, distinct enzymes and reactions, and their main effects in the enzyme superactivity. In our opinion, and despite the high number of studies reporting the use of different methodologies trying to understand the molecular mechanisms in micellar systems since the 90s [15,81–83], the effect of the addition of additives or even co-solvents to the medium should be clarified, since they seem to have a positive influence in the lipase activity [73,84]. Studies considering mixed micelles are poorly explored and thus their use should be addressed in terms of enzymatic behavior and intermolecular interactions [using techniques such as SAXS, Dynamic Light Scattering (DLS), and Small-Angle Neutron Scattering (SANS)], and different mixtures and surfactant ratio.

3.2.1. Ionic liquids and the lipase superactivity

Most literature deals with the application of common surfactants (Table 1) to promote the formation of aggregates (microemulsions, micelles and reverse micelles), but recently, other ionic compounds known as ionic liquids (ILs) with a surfactant character were the focus of studies in the micellar enzymology field. Over the last years, ILs have gained increasing attention as media for performing distinct reactions with (sometimes) remarkable results [85–87]. ILs are molten salts that remain liquid below 100 °C and in many cases, below room temperature. Due to their amphiphilic nature they can dissolve a wide range of compounds and have been considered as a promising alternative to conventional solvents [88]. However, their major interest results from the chemistry at the basis of these compounds to allow the creation of many new families and types of surfactants whose properties can be designed by the appropriate combination of the cation and anion of these compounds. These may lead to an increase of the substrate solubility, improve the enzyme selectivity or enhance the enzyme activity and/or stability [7,22,86,87,89,90]. Most enzymes show a similar level of activity in ILs as in conventional organic solvents, which are considerably inferior to those in aqueous solutions, being this a major limitation for the application of these solvents in biocatalytic reactions. Moreover, the application of enzymes in some ILs is limited by their low solubility, activity and also stability [91,92]. In this context, there is a strong and urgent need to increase the activity and stability of diverse enzymes in ILs. The modification of the solvent environment, namely the formation of micellar systems, represents one of the less studied strategies to improve the enzymatic activity/stability. According to Preiss and co-workers [93], ILs with long alkyl chains and the capacity to form self-aggregates (above the CMC) could represent a future strategy to enhance the kinetics of enzymatic reactions. Recently, Ventura et al. [94] have developed an IL system that is compatible with *Candida antarctica* lipase B (CaLB), which enhance its activity without any enzyme treatment or manipulation of the IL. According to the authors [94], the capacity of long alkyl chain ILs, namely by the application of the 1-decyl-3-methylimidazolium chloride ($[C_{10}mim]Cl$), to promote the formation of micelles by its self-aggregation is the basis for this novel system. For the first time, it was shown a significant increment of the relative lipase activity (up to 6-fold), by the simple application of an aqueous IL solution. These results were obtained without the addition of any surfactant or co-surfactant to promote the aggregates formation. In fact, the number of ILs with surfactant characteristics already identified is large and could be easily enhanced with the quaternary ammonium making a bridge between the common surfactants and these new compounds. This is not, however, the only possible strategy to enhance the activity of enzymes in micellar systems by applying ILs. This emergent class of solvents is also being applied as co-solvent with influence in the enzyme activity [95]. In this case, ILs are acting as co-surfactants and they are added to micellar systems to improve the solubility of the enzyme, or the solute, or the substrate in the media [95], in some cases due to the formation of mixed micelles. Xue et al. [96] proposed an alternative approach investigating the effect of the choline acetate on the activity and stability of a lipase (*Candida rogusa*) in AOT/water/isooctane reverse micelles. The reaction studied in this work was the hydrolysis of 4-nitrophenyl butyrate. The results suggest that at low concentrations of IL, the AOT reverse micelles were not affected in their microstructures. Moreover, it was observed that this IL improved the catalytic efficiency of the lipase and that this activation is due to the effect of the choline-based IL upon the nucleophilicity of the water in the vicinity of the enzyme. The authors also pointed out that the presence of this specific IL (based in a chaotropic cation and a kosmotropic anion) can be responsible for some conformational alterations in the enzyme, thus further justifying the increase of the enzymatic activity. Lai and co-workers [97] have studied the catalytic performance of two distinct enzymes the mushroom tyrosinase and the *Penicillium expansum* lipase considering the addition of 14 distinct ILs. From the results reported, the authors have concluded that the lipase was stabilized and activated in 5.2-fold and 1.4-fold in presence of 0.63 M of choline acetate [choline][Ac] and 0.27 M of trimethylammoniummethyl sulfonate $[NHMe_3][MeSO_3]$,

respectively. Moreover, the higher capacity of the choline structures in the activation of the lipase was proved [97]. The authors also indicated the important role of the IL cations describing the ammonium ILs composed of chaotropic cations (with higher H-bonding capability) and kosmotropic anions as better choices to promote the enzyme superactivity. In this particular case, the authors were capable of to identify a close agreement between the Hofmeister effect of ILs and the enzyme catalytic performance, which they proved by kinetic and thermostability studies. The authors call the attention for different factors and conditions that should be taking into account when ILs are used as co-surfactants, namely the surface pH, active site conformation, and the enzyme catalytic mechanism [97]. Considering the preparation of mixed micelles, Das and co-workers have described also the formation of cationic reverse micelles considering some imidazolium chemical structures. The authors have reported the significant enhancement in performance of interfacially active enzymes, namely *C. viscosum* (CV) lipase and the horseradish peroxidase (HRP) in mixed reverse micelles of cetyltrimethylammonium bromide (CTAB) and imidazolium-based amphiphiles having varying tail lengths [98]. The results showed that the enzymes have a better performance in mixed reverse micelle systems than in the individual cationic surfactants. They described the increase of the enzyme activity, which for the best system was 200% higher for lipase than for systems formed only by CTAB, and even higher for the AOT based system. The alkyl chain length of the imidazolium tensioactive structures was tested and it was concluded that the "exceptional increase in activity with chain length is presumably due to the appropriate positioning of the imidazolium cation at the interface from where it can offer its activating effect to the enzymes by increasing the a-helix content of enzyme", being thus the best results described for compounds with alkyl chains of 14 carbons [98]. Despite the number of publications dealing with the ILs capacity to form microemulsions by their auto-aggregation and the respective CMC determination, few articles are addressing analysis of the type, size and properties of these micelles, depending not only of the ILs concentration and ILs type (alkyl chain length, the cation core and the anion moiety) but in particular considering the enzyme's presence on both types of aggregate bulks, micelles and mixed micelles (when ILs are applied as additives or even co-surfactants). The use of ILs is thus a promising approach in this field. It seems from the results presented that some families and ILs' structures may have the capacity to enhance the enzyme activity, and even the stability, making them excellent alternative surfactants (acting alone) or additives (co-solvent and/or co-surfactant) to increase the yield of reactions promoting a more sustainable production of added-value compounds. In this context, the molecular interactions and the bulk of aggregates should be studied using different methods that may help probing the nature of the intermolecular interactions, namely DLS, SAXS and SANS. We believe that it is necessary to investigate the conformational aspect of these micellar systems, because this is important for the complete understanding of the aggregates formation, mainly when these ILs are used in the micellar enzymology field [17,18,99]. Moreover, the use of distinct structures associated with a proper design of the ILs structures is a crucial concern and should be carried out, since catanionic, dicationic, gemini, and others types of surfactants ILs are being synthesized and have a huge potential for application in this field [100]. A full picture of this phenomenon is thus necessary to make possible to establish heuristic rules in the application of ILs and their surfactant nature to promote the superactivity phenomenon.

3.3. Other enzymes

Superactivity has also been reported for other enzymes. In 1997, Gebicka et al. [101] have shown that the activity of horseradish peroxidase (HRP) in AOT/n-heptane reverse micelles toward 2,2′-azino-bis[3-ethyl-benzothiazoline-(6)-sulfonic acid] was higher by approximately one order of magnitude in comparison with the homogeneous aqueous solution. Mahiuddin et al. [102] have presented the first results of enzymatic activities in a reverse microemulsion medium based on a mixture of an anionic and a cationic surfactants, designated by catanionic microemulsion, where it was possible to observe the superactivity of the HRP. Recently, Mahiuddin and co-workers [19] have suggested that the superactivity of the HRP encapsulated in a cationic reverse microemulsion is correlated with the change in the solvation state of the enzyme active center. The application of water-in-IL microemulsions (composed of AOT/1-octyl-3-methylimidazoliumbis(trifluoromethylsulfonyl)imide)/water/1-hexanol) as the reaction medium for the enzymatic oxidation of pyrogallol catalyzed by HRP was also investigated [103]. The results demonstrated that the rate of HRP-catalyzed reactions in these microemulsions in the presence of this imidazolium-based IL was significantly increased compared with the results obtained in the oil microemulsions (without the IL presence) [103]. The superactivity of HRP together with the soybean peroxidase (SBP) was also assessed in literature [104], by applying single-walled carbon nanotubes hybrid in CTAB reverse micelles. The results seem to demonstrate that the enzymes showed an enhancement in its activity ~7–9-fold, in comparison with its behavior in the aqueous buffer. This enzyme was also studied in what concerns the effect of the unsaturation at the surfactant head.

Elhefnawy et al. [105] recently addressed the influence of the composition and structure of water-in-oil microemulsions on the activity of the Iraqui Turnip peroxidase. In this work, reverse microemulsions composed of chloroform, aqueous buffer, sodium dodecylsulfate, and alcohols of the homologous series from 1-propanol to 1-hexanol were used. From the analysis of the main results, it seems that the enzymatic activity is improved when the alkyl chain length of the alcohol is increased. Moreover, the author states that when the overall concentration of the enzyme is constant, the enzyme catalytic behavior is dependent of the size of the water pool [105].

The hexokinase (HK) enzyme was studied also in the presence of a cationic, an anionic and a nonionic surfactants, namely the hexadecyltrimethylammonium chloride (HTAC), the AOT and octaoxyethylene dodecyl ether ($C_{12}E_8$) [24]. In this study, the enzyme activity was enhanced in 2–3-fold when the HTAC was applied and by comparison with the AOT data. Furthermore, the results showed that the $C_{12}O_8$ reverse micelles where responsible for the best results considering the HK activity, being indicating that the catalytic performance of the enzyme was improved in the presence of the nonionic surfactant when compared with the remaining two surfactants investigated [24].

Furthermore, the thioesterase activity was also studied under the addition of Brij-58, a nonionic surfactant, above its CMC. The main results suggest that the presence of the surfactant has a dramatic effect on the thioesterase activity, being the catalytic activity extended to >60 min and the rate of cyclization (but not hydrolysis) increased up to 6-fold, resulting in a net 150–300-fold increase in cyclic product yields [36]. This significant effect is explained by the interaction between the enzyme and the micelles [36].

Some works using the chloroperoxidase [23] studied the effects of several polyhydroxy compounds and the surfactant AOT on the catalytic activity and thermal stability of the enzyme in aqueous systems. The results indicate that, at 25 °C, the superactivity phenomenon was observed in presence of AOT being the catalytic efficiency of the chloroperoxidase increased by 25%.

The catalase was also investigated using reverse micelles of Brij-30 and micelles of Brij-35 in dodecane, n-heptane, or isooctane [35]. The enzyme only retains the activity in aqueous micellar solutions

of Brij-35. In this study, the catalase activity was found to increase 1.5 times the activity of cytoplasmic glycerol 3-phosphate dehydrogenase [106] and in 1.8-fold the activity of *Bacillus amyloliquefaciens* α-amylase [107].

The activities of cellulases from *Trichoderma reesei*, entrapped in three types of reverse micelles were also studied, using microcrystalline cellulose as the substrate [108]. In this work, the reverse micellar systems were formed by the Triton X-100, AOT and CTAB surfactants in an organic solvent media, respectively. The results indicate that cellulases show superactivity in these reverse micelles with the enzymatic activity in AOT, CTAB and Triton X-100 reverse micelles being increased 5, 7 and 35-fold, respectively when compared with the aqueous system at the optimum conditions. These values were explained by different interactions occurring between the enzyme and the surfactant layer [108]. The use of rhamnolipids to stimulate the cellulase activity was also tested considering the rice straw hydrolysis [104]. In this work it was concluded that, not only the biosurfactant is acting in the cellulase activity increase (this parameter is 115% higher than that of the control sample), but also that the rhamnolipid allows the reduction of the enzyme amount and its recyclability [104].

The α-amylase was studied to evaluate of the effects of surfactants and oxidizing agents in its enzymatic stability [38]. The authors studied the influence of three anionic surfactants, the Triton X-100, Tween 20 and Tween 80 and the anionic surfactant SDS. The results suggest that this enzyme is completely stable when strong anionic surfactants are present and more interestingly, its stability is improved by 120% when H_2O_2 is added [38]. Zeng and collaborators [37] also investigated an amylase, plus a carboxymethyl cellulose enzyme (CMCase), a xylanase and a protease of *Penicillium simplicissimum*, isolated during the solid-state fermentation. The catalytic behavior of these enzymes was investigated considering the addition of the anionic surfactant Tween 80 and a rhamnolipid, considered here as a biosurfactant. The results obtained showed that the addition of the surfactants resulted in distinct effects on the four enzymes and also that the effects of the two the surfactants on the same enzyme were also different. The performances of the enzyme activity were discussed in comparison with a control system and it was possible to conclude that in some specific conditions, the enzyme activities were higher when the surfactants were present than in the control system. It was demonstrated that both surfactants increased the amylase, CMCase and xylanase activities, being the stimulation of xylanase the most prominent. However, the authors also observed that the protease suffered an inhibitory effect, and thus the superactivity of this enzyme was not possible when the surfactants were used [37].

Unlike for α-CT and lipase, the studies for the other enzymes are sparser and not systematic. Trends are difficult to draw based on the limited results available, but they are nevertheless encouraging showing that a large number of enzymes can be activated in micellar systems. An exploration of other enzymes and reactions is now required along with a systematic study aiming at establishing some heuristic rules that allow the application of this technique in systems of industrial relevance.

4. Conclusions and critical analysis

This work reports an overview of the enzyme superactivity induced by micellar systems. Several conditions and structural features were addressed in this review, namely different enzymes, surfactants (not only synthetic, but also biosurfactants, such as rhamnolipids) and reactions. Moreover, the surfactants' concentration and the addition of co-surfactants or additives were also conditions considered in some of the aforementioned studies. Although this phenomenon has been reported for a variety of enzymes, there is still a wide range of enzymes and/or reactions to be attempted. There is a lack of information and studies considering the application of the superactivity phenomenon to reactions with industrial interest, which means reactions producing added-value products with enzymes where superactivity induction has been demonstrated, instead of just the model reactions commonly used on these studies. To induce the superactivity of lipase and cellulase, for example showing a capacity to increase the hydrolysis of vegetable oils for the production of fatty acids, the production of emollient esters (with cosmetic interest), or fermentable sugars (potentially applied in the production of biofuels). We believe that the superactivity phenomenon will require a case-by-case approach and that the determination of the effect of different, common and alternative, surfactants is of utmost interest and importance. However, for a deeper understanding of the superactivity phenomenon, a systematic study considering these reactions with industrial interest, while changing the surfactants type from anionic, cationic and nonionic, and their characteristics, must be carried to identify the main interactions controlling the superactivity phenomenon. For that purpose, a variety of common techniques to evaluate the characteristics of the micellar systems and their impact on the enzyme activity must be used. Here, the same parameters described along this review should be taking into account, due to the variable action of different substrates, reactions, surfactants and even products formed.

In this case, a multidisciplinary approach should be consider for (i) the definition of the reaction and substrate to a better characterization of the micellar system taking into account the presence and absence of the enzyme and (ii) a study contemplating the main interactions controlling the superactivity phenomenon and the reaction performance, for the pairs 'enzyme + tensioactive', 'enzyme + micellar system' to understand the mechanisms promoting the superactivity, by applying the most common techniques previously described [Dynamic Light Scattering (DLS), Small-Angle X-Ray Scattering (SAXS) and Small-Angle Neutron Scattering (SANS)]. In the present review it was identified a clear relationship between the polar head-group size and the alkyl chain length with the superactivity phenomenon, which suggests that other studies should be performed considering surfactants with bigger head-groups, namely gemini-surfactants (composed of two similar connected head-groups) and substituted by longer and different alkyl chains. Based on these results, a proper design of the surfactants or bio-surfactants can be achieved to maximize the enzyme catalytic performance and significantly improve the yield of the reactions. The results reported in this review shown that while several studies of enzymatic reactions in micellar systems have been published, they focused on fundamental aspects and in the measurement of the catalytic activity, but the product is rarely focused in these studies. In fact, the lack of works addressing the products, and not just the phenomenon or its mechanism, is an important limitation because the products isolation and the recovery and reuse of the surfactants is difficult due to the complexity of the medium with surfactants, co-surfactants or additives and, in this context, it is imperative to address the isolation of the reaction products, principally considering the reactions with industrial relevance.

Considering the superactivity inductors, ILs represent a potential vehicle toward novel methodologies promoting the enzyme activation. In this context, it seems that the understanding of the influence of the IL structure, not only considering the alkyl chain length and the use of different anion moieties and cation cores, but also the application of dicationic, gemini and catanionic structures is of high importance, as previously discussed. In fact, the synthesis of new ILs classes is now gaining interest, not only in terms of having new structures to evaluate, but mainly because the IL synthesis is starting to consider their "designer solvent" characteristic,

taking into consideration the final applications and, their performance in those applications. Also in this field, we believe that the proper design of ILs should be considered and the structure/properties/application combination approached ensemble in the design of new works. Furthermore, the analysis of the surfactant and IL aggregation arrangement and their correlation with the superactivity phenomenon is of great interest. In this context, different works should be planned considering the specific analysis of the aggregates formed by the different tensioactive ILs and no less important, the scientific community should pay attention, not only to the conceptualization and detailed comprehension of this phenomenon, but also and very important, we need to look forward and trying to cross this concept, with the industrial application. In this sense, the studies in this area should not be carried only at a lab scale. In fact, their scale-up considering different enzymes catalyzing different reactions is required for the industrial implementation of this approach in the production of the desired added-value compounds. In this context, different experiments should be carried out, not only in what respects the choice of the best conditions identified at lab scale, but also the technological viability, process sustainability and environmental impact of the whole process and products and finally, the economic analysis where the technological costs (equipment, reagents, human resources, infrastructure, solvents and surfactants recyclability or recovery, and products purification) and environmental impact (concerning the raw materials, aqueous effluents, and nature of the solvents used) are crossed with the products benefits (having in mind the final price of the target products and/or the intermediate products).

Acknowledgements

This work is financed by FEDER through Programa Operacional Fatores de Competitividade – COMPETE and national funds through FCT – Fundação para a Ciência e Tecnologia within CICECO project - FCOMP-01-0124-FEDER-037271 (Refª. FCT PEst-C/CTM/LA0011/2013) ad for the project PTDC/AAC-AMB/119172/2010. The authors also acknowledge FCT for the post-doctoral and doctoral grants SFRH/BPD/79263/2011 and SFRH/BD/85871/2012 of S.P.M. Ventura and T.E. Sintra, respectively.

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