SCIENCE CHINA
Chemistry

• REVIEWS •

May 2011 Vol.54 No.5: 715–723
doi: 10.1007/s11426-011-4266-2

Effects of surfactants on enzyme-containing reversed micellar system

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Received July 2, 2010; accepted September 6, 2010

With the development of colloid interface and enzyme technologies, enzyme-containing reversed micellar system has been receiving much attention in bioseparation and bioconversion. Because of its high efficiency, it has brought new opportunities for the development of molecular biotechnology. Reversed micelles represent nano-sized aqueous droplets stabilized by surfactant amphiphiles inside the bulk organic solvents. The entrapped enzymes have enhanced activities under those conditions as suited in the lipid bilayers of biological membranes. The fundamentals of enzyme-containing reversed micellar system are described in this paper, with special emphasis on the effects of surfactants varying in concentrations and structures. The latest study progress on the surfactants application in enzyme-containing reversed micelles is reviewed. The introduction of novel functional surfactants in micellar enzymology and their future development are also discussed.

reversed micelle, enzyme, surfactant concentration, surfactant molecular structure, enzyme-containing reversed micellar system.

1 Introduction

The investigation of enzyme-containing reversed micellar system is a novel aspect of colloid and interface chemistry and biotechnology. The reversed micelle is formed by surfactant amphiphiles self-aggregated in the bulk apolar organic solvents. The surfactant molecules assemble themselves with the polar head to the innerside and the apolar tail in contact with the organic solvent. This self-aggregation only occurs when the surfactant concentration is above the critical micelle concentration (CMC). Because of the formed polar cores, reversed micellar system allows nanometer-sized aqueous droplets stabilized in it. The process of the enzyme solubilization in reversed micelles results in the formation of enzyme-containing reversed micellar system [1–3]. The existence of reversed micelles was first reported by Hoar and Schulman [4], who named it "oleopathic hydromicelle". Meanwhile, Hanahan [5] and Misiorowski *et al*. [6] found that many lipases retain high activity and even show "superactivity" when they are stabilized in reversed micelles. Then in 1977, the possibility of protein extraction with reversed micelles was pronounced for the first time by Luisi *et al*. [7] and his partners, after they found that α-chymotrypsin can be solubilized in the organic solvent which contains surfactant. In the same year, the first study on the catalytic activity of peroxidase and of chymotrypsin entrapped in hydrated reversed micelles formed by AOT was carried out by Martinek *et al*. [8], whose pioneering work stimulated extensive research on "micellar enzymology".

Reversed micellar system is optically transparent and of thermodynamically stabilization, while it is also a microheterogeneous medium which represents high dissolution of single reversed micellar aggregates. It provides a

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mesoscopic environment for reactions in it. In recent research on nanoscience and nanotechnologies, reversed micelles as nanoreactors have many advantages [9, 10]. As recognized, the nano-sized hydrophilic droplets are dispersed in a hydrophobic solvent and stabilized by surfactant molecules which self-assemble at water-oil interface. Therefore, the micro-compartmental structure is formed. Meanwhile the inner system of reversed micelles is suggested to be a suitable mimetic environment such as in the living cell [11]. This explains the "superactivity" of enzymes in it. As a result, the problem of extending the lifetime of extracellular enzymes as well as making them best suited for non-native reacting environment was approached [12, 13]. Recently, the superior activity of hydrophobically adsorbed enzymes on to single-walled carbon nanotubes (SWNTs) in cationic reversed micelles was reported by Das *et al*. [14]. According to this research, both horseradish peroxidase (HRP) and soybean peroxidase (SBP) adsorbed onto SWNTs endured a notable loss in secondary structure and catalytic activity. However, the enzyme-carbon nanotube hybrid in CTAB reversed micelles showed ~7–9-fold enhancement in activity compared to that was in aqueous buffer. Also, it showed the activity was achieved ~1500–3500 times higher than that in aqueous-organic biphasic mixtures. The results gave a successful example of functional nano-materials with biomolecules. At the same time, the great potential of reversed micelle in activating supreme ability of enzymes was observed.

As an individual component in forming the reversed micellar structure, surfactant was found to play essential roles in the enzyme-containing reversed micellar system. Some researchers made efforts to this field and gained some major progresses. However, more works should be devoted to that. This work mainly focuses on the effects of the surfactants on enzyme-containing reversed micellar system, with special emphasis on effects of the concentration of surfactants and their molecular structures. The aim of this study is to provide guidance in surfactant quantitative optimization and qualitative determination during practical manipulations. The important researches in this field as well as the further trends are also discussed.

2 Fundamentals of enzyme-containing reversed micellar system

The enzyme-containing reversed micellar system is thermodynamically reversible. The localization of enzymes in the system depends upon the protein hydrophilicity/hydrophobicity (Figure 1). Hydrophilic enzymes (such as chymotrypsins) are located in the inner water cavities of micelles avoiding contact with the organic solvent. A surface-active enzyme (such as a lipase) can interact with the inner micellar interface, while molecules of hydrophobic enzymes (for example, of xanthine oxidase, atpases, CO dehydrogenase or alkaline phosphatase) can localize in the hydrophobic region of the micelles interface. The inclusion of enzymes in reversed micelles occurs spontaneously. It can be dealt with adding a lyophilized enzyme preparation into a reversed micellar system, or injecting a stock enzyme solution into an organic solvent containing surfactant. Shaking or mixing will help to establish the equilibrium [11, 15].

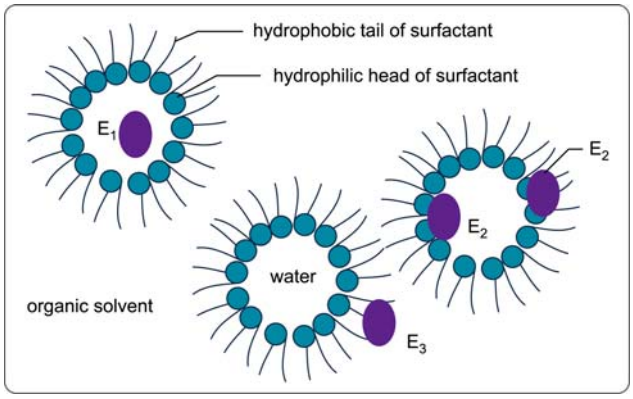

Figure 1 Schematic representation of the enzyme-containing reversed micellar system (E_1: hydrophilic, E_2: surface-active, and E_3: hydrophobic enzymes) [11, 15].

The surfactant interface can in principle protect the entrapped enzymes against the inactivation by bulk organic solution. As a result, the reversed micellar system showed a great potential in facilitating the enzymatic catalysis in it. Also the system is of technological interest in bioseparation and purification of proteins from fermentation broths [2, 3].

Enzymes in reversed micellar medium often show completely different catalytic properties compared with those observed in aqueous solutions. Actually, the hydrophilic substrates can solubilize into the nano-sized water cavities, and then come into the enzymes. The water-insoluble substrates are easily dissolved in the organic solution or adsorbed onto the surfactant membrane. The catalytic reactions proceed with the effective mass transfer performance [16].

The enzyme purification via the reversed micellar system reveals a challenge to the liquid-liquid extraction technology's requirements. The Figure 2 represents the process of enzyme purification via a reversed micellar system. There are usually two steps during such a purification process. The forward extraction proceeds to transfer the protein molecules from fermentation broth into the reversed micelles. While the backward extraction would allow the target proteins recovered in another liquid phase, or be precipitated out from the reversed micelles in a solid state. Reversed micellar system extraction is a new approach for the bioactive molecules separation and purification. It has been receiving a sustained attention in the medicine, food projects and biological engineering. Compared with the conventional purification methods, the extraction via reversed micellar system

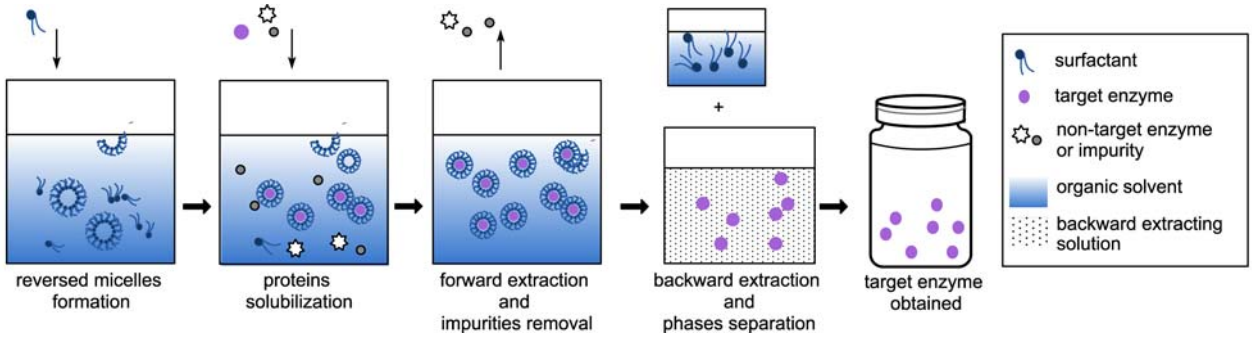

Figure 2 Scheme of the enzyme purification via reversed micellar system.

has the advantages of less operating procedures, more facility in manipulation, lower cost, higher specificity and capacity for preventing enzyme denaturalization [3, 17–22].

It is accepted that the enzyme-containing reversed micellar system has two main advantages as follow [11]. First, it is a microheterogeneous system, which provides means for easy dissolution of both hydrophilic and hydrophobic substances in it. In other words, the molecule of the solubilized enzymes can in principle come into contact with water-soluble, surface-active and water-insoluble substrates. Second, reversed micellar system ensures a strict nanocompartmentalization of solubilized macromolecules, thus permitting a controlled build-up of subunit structures. In particular, surfactant aggregates form as a reversed micellar layer which protects the entrapped enzymes against deactivation by the bulk organic phase. Meanwhile the surfactant shell divides the solution into the nano-sized water droplets and organic phase. This amphiphilic interface and the inner water pool can be adjusted by the parameters of the individual factors, and finally, the characteristics of the entrapped enzymes as well as their behaviors can be optimized through the subunit structures adjustment.

3 Effects of the surfactant concentration on enzyme-containing reversed micellar system

Surfactant concentration may affect the physicochemical properties of the reversed micellar microaggregations, and change the solubilization or activity of the enzymes. In respect of enzymatic catalysis, surfactant concentration may even influence the distribution of the substrate.

3.1 Effects of the surfactant concentration on the size, shape and microscopic properties of the reversed micellar microaggregates

The size of the micellar microaggregates depends on the inclusion of water content and surfactant concentration [11], therefore the molar ratio of water to surfactant concentration (W_0) is usually adopted as a measurement. If the concentration ratio of water to surfactant remains constant, the micelles' size will not change (Figure 3B). When the water content maintained, as the concentration of surfactant increased, the micelles become smaller (Figure 3C). On the contrary, the higher water content in the system, the larger is the radius of the micellar particle (Figure 3A).

The reversed micellar aggregates are thermodynamically stabilized other than being an absolutely rigid matrix. Depending upon the individual components, the microaggregates associate themselves with a spherical, ellipsoidal or cylindrical shape. The water inside the reversed micellar system can be divided into two parts according to their different localizations and physical properties. The bulk water in the droplet core or dissolved in the organic solvent is considered as "free water", while the "bounded water" is tightly in contact with the surfactant layer. Thereby the reversed micelle solvent is non-aqueous liquid. When the surfactant concentration is low, the number of microaggregates is insufficient to prevent the activity of entrapped enzymes. Under that condition, the water dissolved in such a system is almost in normal property. In contrary, if the surfactant concentration is too high, the amphiphilic molecules will bond with each other and probably result in denaturalization of enzymes [23]. Therefore the right concentration of the surfactant in reversed micellar system is important.

In conclusion of the previous researches, it can be considered that, over a wide range of surfactant concentration,

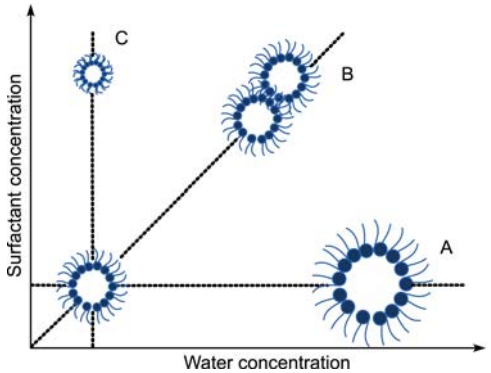

Figure 3 Effect of the content of water and surfactant on a reversed micellar system [11].

variations of the surfactant concentration only affect the number of micelles, without changing their properties and/or their size and shape [15]. The strict limitation of this effect has not been established by far. In addition, surfactants of different natures will bring up multiple influences.

3.2 Effects of the surfactant concentration on the enzymes

The concentrations of surfactants can also affect the amount of solubilized enzymes and their catalytic actions. Commonly, the increase of the surfactant in the organic phase will lead to an increase of the protein solubilization, as the increase of surfactant aggregates and/or of their size. However, those changes will not always happen. According to Liu *et al*. [21], in the extraction of nattokinase by reversed micelles, as the AOT concentration increased from 50 to 200 mM, protein recovery increased slowly and the activity recovery didn't show significant increase. Another case based on the experiment of enzymatic hydrolysis of carboxymethyl cellulose in different reversed micellar systems, we [24] found that the peak conversion rates were all obtained when the surfactant concentration was at 1 CMC of their each, irrespective of the substrate concentration and other reacting conditions.

Most of the enzymes are divided into two groups by Martinek *et al*. [11] according to their behaviors of interacting with surfactant in the reversed micellar system. The first group of enzymes should not depend on the surfactant concentration, if the value of W_0 is kept constant, including α-chymotrypsin, trypsin, alkaline phosphatase and lipoxygenase, etc. The second group of enzymes include peroxidase, acid phosphatase, laccase and prostaglandin synthetase, whose activity is confirmed to strongly depend on the surfactant concentration. Those enzymes from the second group are characterized by the presence in their molecules of 'anchoring groups of different nature'. It is suggested that such groups may be of hydrophobicity and capable of interacting with micellar membrane. It thus causes the dependence of catalytic activity on the surfactant concentration [25]. The validity of this suggestion was confirmed by Kabanov *et al*. [26], who carried out the comparative studies of catalytic action of α-chymotrypsin in the native state and after covalently modified with stearoyl residues. The results demonstrated that when solubilized in AOT- octane reversed micelles, the native α-chymotrypsin didn't show any dependence of catalytic activity on the surfactant concentration at a fixed value of W_0. However, in the case of the hydrophobized α-chymotrypsin, a profound dependence of catalytic activity on the surfactant concentration was observed, showing a decrease of the catalytic rate constant (K_{cat}) with the surfactant concentration increment. Still the effects of the surfactant concentration on the distribution and catalytic behaviors of enzymes in reversed micelles are complicated. It needs further researches focused on.

3.3 Effects of the surfactant concentration on the substrate distribution in catalysis

Another reason of the dependence of the enzyme activity with the surfactant concentration is the substrate distribution [15]. The catalytic activity of the enzyme is in relation with the substrate which coming into contact with them. The substrate behavior is affected by the surfactant concentration when it distributes between micellar interface and the organic phase. With regards to the kinetics of enzymatic catalysis in reversed micelles, a pseudophase model [16] proposed considers that a part of the overall substrate remains in the micellar sub-phase in close contact with the surfactant due to the electrostatic affinity or hydrophobic adsorption. The increase on surfactant concentration will decrease the substrate accessed by the enzyme, and consequently reduce the value of K_{cat} [27].

On the other hand, it was reported by several researches that less influence of the surfactant concentration was observed on the hydrophilic substrate, compared with a hydrophobic one. The above phenomena may be attributed to the molecular thermodynamics during the reacting process. While the collision theory gave an illustration in this view, it explained that in some systems it has been observed an increment in rate with the surfactant concentration. Commonly the reversed micelles collide 10^9–10^{11} times every second, while the fast and frequently exchanges among the entrapped substances are proceeding. The exchange rates of the hydrophilic substrates are in the range of 10^6–10^8 mol, which represents an exchange may occur during 1000–10000 times of collision. Otherwise, the exchange rates of the hydrophobic substrates are larger [28]. At higher surfactant concentrations, the reversed micelles collide more frequently as their large population. As a result, the diffusion and exchange of the molecules in the system are enhanced [3, 29].

4 Effects of the surfactant molecular structure on enzyme-containing reversed micellar system

Surfactant amphiphilic molecule consists of a hyrophobic tail usually formed by alkyl chain and together with a hydrophilic polar headgroup. The surfactants structural characteristics determine their physicochemical properties and their functions. The nuclear magnetic resonance spectrum (^{1}H NMR) indicated that most of the reactions take place in the surfactant interface of the micellar system. Therefore, the electrostatic property as well as the molecular structure of the surfactant membrane is very important.

4.1 Electrostatic interactions

The electric charges of surfactant head-groups depend on their ionic structures of molecules.

The lipases and α-chymotrypsin catalyzed hydrolytic reactions in reversed micellar systems have been extensively investigated. In most cases, it has been observed that the hydrolytic activity is much higher in AOT reversed micelles than in cationic, non-ionic or zwitterionic-based systems under the same condition. The xanthine oxidase catalytic behaviors with several substituted benzaldehydes in water and in reversed micelles of different charges (AOT, DTAB and Triton-X 100) have been studied by Bommarius *et al*. [30]. It was found that the catalytic efficiency follows the order: DTAB > Triton-X 100 > water for hydroxybenzaldehydes with hydroxyl groups in meta and para positions. Kuwahara *et al*. [31] reported the catalytic actions of hexokinase (HK) in reversed micelles of AOT (anionic), HTAC (cationic) and $C_{12}E_8$ (nonionic) surfactants. Results indicated that catalytic activity of HK in HTAC was 2–3 times higher than that in AOT reversed micellar system. Moreover, in $C_{12}E_8$ reversed micelles, the HK activity was much higher than those in the other systems. It was proposed that the highly charged inner surfaces of AOT and HTAC reversed micelles constrained the HK catalytic activity.

The addition of nonionic surfactants is able to modify the interface of cationic reversed micelle. Through synergistically interacting with the cationic surfactant, nonionic surfactant can effectively reduce the positive charge density at the micellar interface. Therefore, they markedly increase the flexibility of interface simply by reducing the chance of cationic inhibition at the active site of the enzyme, and thereby improving the enzyme activity (Figure 4). A *Chromobacterium viscosum* lipase (CV-lipase) was employed in reversed micellar system according to the experiment carried out by Shome *et al*. [32]. The activity of the CV-lipase was determined in the mixed reversed micelles of CTAB combined with several nonionic surfactants (Brij-30, Brij-92, Tween-20, and Tween-80) dissolved in the isooctane/*n*-hexanol solvent. The *p*-nitrophenyl-*n*-octanoate was used as the substrate. The catalysis at different z ([cosurfactant]/[surfactants]) values, pH 6, 25 °C across a varying range of W_0 ([water]/[surfactants]) was evaluated. It was shown by the results that the lipase activity was improved maximum up to ~200% in mixed reversed micelles compared to that in CTAB/water/isooctane/*n*-hexanol alone. Interestingly, this observed activity was even higher than that obtained in AOT reversed micellar system. While another surface-active enzyme, HRP was also examined and exhibited the similar trend as observed for lipase.

The orientation of the substrate in electrostatic field of micelle is proved depending on the sign of surface potential. Ermakova *et al*. [33] studied the effect of surface potential of reversed micelle on enzyme-substrate complex (encounter complex, EC) formation by Brownian dynamics simulation. The research pointed out that the negative potential of micelle increases the probability of EC formation and the positive potential decreases it.

4.2 Effects of the surfactant head-group hydrophilicity

The hydrophilicity of the surfactant head-group is considered affecting the concentration of water $[H_2O]_i$ at inner surface of micelle, and consequently influencing the enzyme activity. However, it is distinct that in reversed micellar system of cationic surfactant, as the catalytic efficiencies of surface-active enzymes cannot be regulated simply by changing the W_0 due to unchanged $[H_2O]_i$. It figured out why the enzyme activity was usually lower in cationic micellar systems compared with that in others. Meanwhile, the introduction of hydroxyethyl groups is confirmed to increase the enzyme activity in CTAB reversed micelle. The

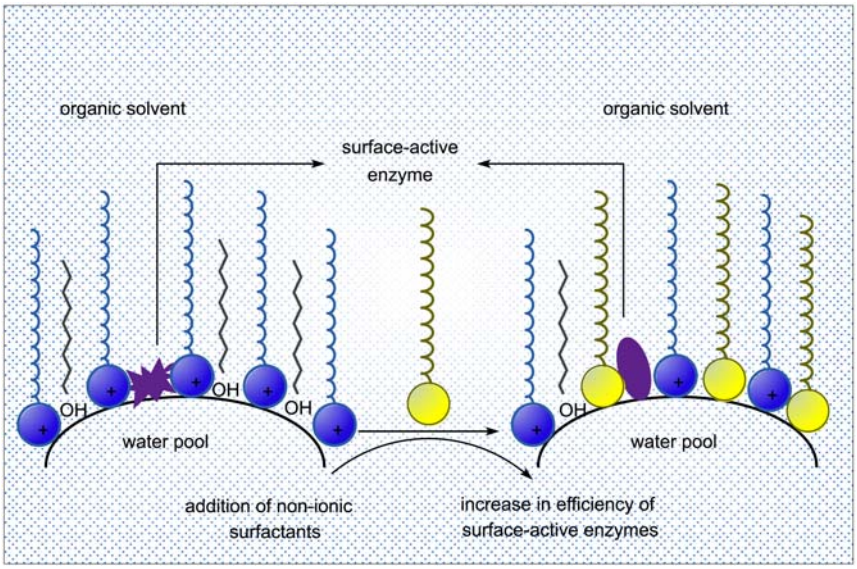

Figure 4 Effect of nonionic surfactant on enhancement of the enzyme activity at cationic reversed micellar system [32].

enhancement is attributed to the increment of water content, as the added hydrogen bonding ability of the hydroxyl groups. Moreover, the catalytic efficiency of enzyme in this hydroxyethyl groups modified CTAB reversed micelle was as higher as it performed in AOT-based systems [34, 35]. In our previous studies [36–38], we have revealed that the adsorption of surfactants on microbials would changed the cell surface lypohydrophilic property, and those effects were of difference in dependent of surfactants hydrophilic structures. Thus it is proposed that the interactions between surfactant hydrophilic head and the enzyme surface chemical groups are significant.

4.3 Effects of the surfactant head-group size and flexibility

The increase in surfactant head- group size allows the enzyme to attain a flexible conformation as well as increase in the local concentration of enzyme and substrate. According to a research of lipase in cationic reversed micellar systems by Das *et al*. [35], the subsequent replacement of three methyl groups of CTAB with hydroxyethyl (series I), methoxyethyl (series II) and *n*-propyl (series III) groups were adopted, while the hydrophilicity at the polar head of this study was gradually reduced from series I to series III. It was shown by the results that the lipase activity was markedly higher in series II relative to their more hydrophilic analogues in series I. In addition, the activity represented was comparable in both series I and III, though the hydrophilicity was drastically different. The data demonstrated that the head-group area per surfactant is almost similar for comparable surfactants of both series I and III, but distinctly higher in case of series II surfactants. Thus, the author concluded that the enzyme activity in reversed micelles was largely regulated by the surfactant head-group size, which plays the dominant role over the hydrophilicity. Dasgupta *et al*. [39] also corroborated the primarily regulation of enzyme in micellar system is the head-group size which increases the space at interface.

Actually, the enzyme activity enhancement according to Das *et al*. [34] was not only because of the increment of the hydrophilicity at the interface by hydroxyethyl groups' introduction, but also owing to the changes of surfactant head-groups flexibility. It has been found that the flexibility of the surfactant head plays an important role in modulating the enzyme efficiency by altering the head-groups size/area. Accordingly, the activity of enzyme was found unchanged in the different reversed micelles originating from the rigidity at the surfactant head, despite the hydrophilicity was greatly enhanced with the hydroxyethyl moieties substitution. In contrast, the enzyme activity was found increased with the improvement of geometrical flexibility at the surfactant head, without changing the hydrophilicity at the interface [40].

4.4 Effects of the surfactant head-group unsaturation

Unsaturated head-groups introduced will decrease the enzyme activity due to the content loss of α-helix. Circular dichroism (CD) spectra analysis of enzyme in reversed micelles revealed that the ellipticity in the far-UV region increases with increasing unsaturation of the surfactant molecule. For the first time, Debnath *et al*. [41] investigated the influence of unsaturation at surfactant head on enzyme activity in reversed micellar system. The lipase and horseradish peroxidase (HRP) were tested in cationic reversed micelles. Similar trend in deactivation of enzymes was observed in the presence of acyclic unsaturated substitution at the surfactant head in comparison to the cyclic unsaturation. The study also corroborated the enzyme activity decreases with increase in degree of unsaturation at the interface of micelle. Moreover, it is important that the inhibition of enzyme by unsaturation overwhelms the activating effect of head-groups size of surfactant.

4.5 Effects of the surfactant hydrophobic tail length

Most of the surfactants with single chain structures showed a linear correlation between the number of carbon atoms and characteristic in solution. It has been found [42] that the enzyme activity increased in reversed micellar system with the surfactant alkyl chain length increment, irrespective of the nature of the surfactant head-group. The increase of hydrophobic tail length probably increased the "space" of interface in the system, and as a result the local concentration of enzyme and substrate increased. It is assumed that the augmented reacting interface can obtained either by increasing the chain length or improving the head-group size. However, the kinetics studies illustrated that the increase in chain length will not always bring on the increment of catalytic rate. Thus, the change in partition of substrate and enzyme is one of the reasons for enzyme activity improvement. Further efforts should be made to clarify if interfacial area is augmenting with increasing tail length resulted from chain folding, then to corroborate the possibility of higher substrate and enzyme concentrations at the augmented interfaces, and finally, to confirm that the changes in the enzyme secondary conformation at the surface.

5 Developments of novel functional surfactants and their further trends and application in enzymology

Up to date, the surfactants applied in reversed micellar system are almost limited to chemical synthetic surfactants, such as the AOT, CTAB, Trioctylmethylammonium chloride (TCMAC), Phosphatidyl ethanolamine (PTEA) and Phosphatidic acid (PTA), etc. The AOT is the most widely used one. Seeking for better approaches to problems of re-

versed micelles is an important trend in micellar enzymology, while novel functional surfactants must be developed to meet the imperative needs for reversed micelle optimization and application.

It has been shown that the presence of nonionic surfacetants is in favor of enzyme activation in reversed micellar system. However, most of the commercially available nonionic surfactants can't be used in reversed micellar system construction alone. In other words, they usually need cosurfactants such as straight chain alcohols to achieve a stabilized water-in-oil microemulsion. However, the size of the micelles they formed can be tuned only in a narrow range [43, 44]. Considering that the activity of enzyme in reversed micelles is influenced by the water cavity size, high levels of alcohols introduction are the inhibitors of many enzymes. A novel functional nonionic surfactant *N*-gluconyl glutamic acid didecyl ester (GGDE) was synthesized by Zhang *et al*. [45]. The GGDE has two hydrophobic hydrocarbon tails and was used to form the GGDE/TritonX-100–cyclohexane–H_2O reversed micellar system, in which the LiP catalyzed oxidation of veratryl alcohol (VA) was studied. Results showed the catalytic efficiency of LiP in this reversed micelle was 40 times higher than that in the AOT reversed micelle, with the optimized conditions of reversed micellar size, buffer pH and concentration of H_2O_2.

The latest research [46] indicated that the water-soluble surfactant ionic liquid (IL) 1-alkyl-3-methyl imidazolium bromides can be combined with CTAB. The efficiency of the trypsin in such a mixed reversed micellar system was markedly enhanced compared with that observed in only CTAB without microemulsion. The best performance was obtained in the presence of IL with a suitable tail length, for example in the 1-ethyl-3-methy imidazolium bromide (EMIMBr)/CTAB reversed micellar system. The EMIMBr has been proved to offer its activating effect to the trypsin by improving the nucleophilicity of water in the vicinity of the enzyme through hydrogen bonding, and also by preserving the secondary structure in the larger-sized reversed micelles.

The widely used of chemical surfactants, however, will inhibit the enzyme validity and even bring on pollution in environment. Biosurfactant originated from microbial, animal or vegetal metabolism represents the natural surfactant which is environmental-friendly due to its low toxicity, biodegradability, ecological compatibility and high efficiency [47–49]. Biosurfactants application can effectively avoid the contaminated accumulation that definitely caused by unnatural reagents, and thus meet the technic safety and environmental sustainability. It has been proved that biosurfactants can stimulate the secretion of microbial extracellular enzymes and accelerate the enzymatic catalytic bioconversion [50–54]. However, few efforts have been made in reversed micellar system based on biosurfactants. Recently, there are some researchers [55–57] put forward on synthesis of silver nanoparticles in reversed micelles stabilized by biosurfactants. They discovered that the enhanced stability of nanoparticles in such reversed micellar systems was obtained. The above researches demonstrated the feasibility of biosurfactants applied in reversed micellar systems to a certain extent. For the first time, we [24] introduced the rhamnolipid biosurfactant into the reversed micellar system, and focused on the catalytic behaviors of cellulase in the "rhamnolipid/isooctane/*n*-hexanol/water" reversed micellar system. Data from this research showed that the rhamnolipid was more effective than the other three surfactants (CTAB, SDS and Tween 80) under the same conditions.

Otherwise, biosurfactants are of plentiful varieties and easily produced in nature, they must have great potential in enzyme-containing reversed micellar systems, and worth for further development in this field.

6 Conclusions

As the enzyme-containing reversed micellar system is microheterogeneous and of multiple phase composition, the phase behaviors in it are complicated, while the interaction between the components is indefinite. More surfactants should be introduced into such micellar systems, and further efforts should be made to promote the industrialization of the key technologies in this field. As the development of novel functional surfactants are actively carried out, especially the progresses in biosurfactants as well as the nanotechnologies [58], more extensive applications of the enzyme-containing reversed micellar system are expected. Further researches on micellar enzymology will be completed such as figuring out more mechanisms in reversed micellar system.

This work was supported by the National Natural Science Foundation of China (50978087，50978088 and 51039001), the Program for Changjiang Scholars and Innovative Research Team in University (IRT0719), the Hunan Provincial Natural Science Foundation of China (10JJ7005) and the Hunan Key Scientific Research Project (2009FJ1010).

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