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Review

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

Tânia E. Sintra, Sónia P.M. Ventura**, João A.P. Coutinho*

CICECO, Departamento de Química, Universidade de Aveiro, 3810-193 Aveiro, Portugal

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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.

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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

* Corresponding author. Tel.: +351 234 401507; fax: +351 234 370084.
** Corresponding author. Tel.: +351 234 370958; fax: +351 234 370084.
E-mail addresses: spventura@ua.pt (S.P.M. Ventura), jcoutinho@ua.pt (J.A.P. Coutinho).

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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.