Kinetics of reactions catalyzed by enzymes in solutions of surfactants

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Abstract

The effect of surfactants, both in water-in-oil microemulsions (hydrated reverse micelles) and aqueous solutions upon enzymatic processes is reviewed, with special emphasis on the effect of the surfactant upon the kinetic parameters of the process. Differences and similarities between processes taking place in aqueous and organic solvents are highlighted, and the main models currently employed to interpret the results are briefly discussed.
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1. Introduction

Since 1977, after the pioneering work of Martinek et al. [1], micellar enzymology has arisen as a new physicochemical line of research to approach problems in molecular biology. In fact, in the living cell, enzymes exert their function in microheterogeneous environments interacting with, or being incorporated into, membranes. Even in the cytoplasm, water plays structural and functional roles, besides being the principal component, as in laboratory assays [2]. In vitro systems that make use of the self-assembly of surfactants in aqueous solution (forming micelles or vesicles) or in organic solvents (forming reverse micelles, w/o microemulsions or reverse vesicles) are used to mimic "in vivo" conditions.

Micellar enzymology is devoted to the study of reactions catalyzed by enzymes in solutions of surfactants. Biocatalysis in apolar organic solvents is gaining increasing importance, both for academical studies and industrial applications [3,4]. The field has attracted the interest of many researchers and the relation of micellar enzymology to subjects such as bioorganic synthesis [5], and its potential applications in industrial biocatalytic processes [6,7], biodegradation of phenolic environmental pollutans [8]. The field of enzyme solubilization in hydrocarbon solvents by reverse micelles have been reviewed [9–11], but several physicochemical aspects are still not totally understood. Thus, in spite of more than two decades of intensive work, several problems remain under study in this ample and multifactorial subject. From a kinetic point of view, several aspects must be taken into consideration, such as:

(i) the microenvironment in the surroundings of the enzyme and the substrate [12], and the structural effects resulting from the enzyme-surfactant interaction [13,14];

(ii) the partitioning of the substrate between the micelles and the external solvent [15,16],

(iii) the effect of the surfactant concentration upon the kinetic parameters [16,17];

(iv) the effect of water content on the catalytic behaviour in reverse micelles [18];

(v) the substrate concentration scale that has to be employed in the evaluation of concentration dependent kinetic parameters for a meaningful comparison of the kinetic behaviour of the enzyme in the reverse micelle system [19,20];

(vi) the effect of additives [21,22];

(vii) the effect of changes in the internal [23,24] or the external solvent [21] in reverse micelles;

(viii) the mode of enzyme-surfactant interaction (cooperative, anticooperative) [25];

(ix) the effect of denaturants [26–30]; and

(x) the dependence with the surfactant type [31–35].

This review deals with the general aspects of enzymatic catalysis in solutions of surfactants focusing on the behaviour of enzymes that follows a Michaelis–Menten mechanism. Surfactants in aqueous solution, as well as in organic solvents, are considered. Emphasis is given to results reported after

1995 since former data have been exaustively reviewed by Maitra [10].

2. Surfactants, micelles and reverse micelles

Surfactants are amphiphilic molecules that posses both hydrophilic and hydrophobic parts. The hydrophilic moiety is called the head and the hydrophobic part the tail (or tails). The hydrophobic part may consist of a single chain or it may have up to four chains. The head can be a charged or uncharged polar group. According with the nature of the head groups the surfactants are classified into anionic, cationic, non-ionic and zwittterionic (amphoteric). These types of structures are capable of spontaneously aggregate in aqueous solutions to form micelles (M) and, in organic solvents, reverse micelles. In the present contribution, the term reverse micelle (RM) is applied to aqueous droplets stabilised by a surfactant, dispersed in a continuous organic medium. The term w/o microemulsion will be used in cases that the presence of cosurfactant is necessary to form the microaggregate, i.e. for surfactant/cosurfactant/oil/water systems. In these systems the cosurfactant will be considered as an additive.

Normal micelles, which will be called micelles (M), are depicted as aggregates of surfactant molecules with the hydrophilic heads oriented towards the dispersing solvent (generally water), and the hydrophobic tails oriented towards the inner part of the assembly (the micellar core). Micelles appear as the dominant form above the so-called critical micelle concentration, CMC, but free surfactant is also present in the system as monomer. The aggregation number (the average number of surfactant molecules in a micelle) is dependent on the surfactant type and its concentration. Typically, at low surfactant concentrations (below ca. 0.1 M), micelles are formed by 100–200 surfactant molecules and the aggregation number is nearly independent on surfactant concentration, ie., an increase in surfactant concentration only leads to an increase in the number of micelles [36]. The field of micellar catalysis, which explores the effect of M on the kinetics of chemical reactions, is well known and stresses the importance of these microheterogeneous media, particularly in organic reactions [37]. Also, these media have been used in enzymatic reactions, where important effects can be observed even at surfactant concentrations below the corresponding CMC. Some surfactants that are commonly used in enzymatic studies in micelles, are summarized in Table 1.

Table 1
Commonly used surfactants for the formation of micelles in studies of enzymatic reactions

Surfactant	Type
Sodium dodecylsulfate (SDS)	Anionic
Dodecyltrimethylammonium bromide (DTAB)	Cationic
Cetyltrimethylammonium bromide (CTAB)	Cationic
Cetyltriethylammonium bromide (CTEAB)	Cationic
Cetyltripropylammonium bromide (CTPAMB)	Cationic
Cetyltributylammonium bromide (CTBAM)	Cationic
Myristyldimethylammonium propanesulfonate (MDAPS)	Zwitterionic
Triton X-100	Nonionic
Polyoxyethylene 9 lauryl ether (PO9)	Nonionic