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Journal of Molecular Catalysis B: Enzymatic 31 (2004) 83–85
JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC
www.elsevier.com/locate/molcatb

Distinct effect of a cationic surfactant on the transient and steady state phases of 2-naphthyl acetate hydrolysis catalyzed by α-chymotrypsin

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Received 9 January 2004; received in revised form 3 May 2004; accepted 15 July 2004
Available online 17 September 2004

Abstract

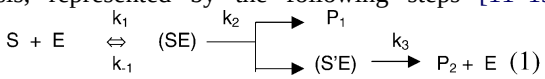
Results obtained on the effect of addition of dodecyltrimethylammonium bromide (DTAB) upon the α-chymotrypsin (α-CT) catalyzed hydrolysis of 2-naphthyl acetate (2-NA) under steady state conditions for the acyl–enzyme intermediate are compared with those previously obtained in the transient (pre-steady state or "burst") phase. It is found that, while in the transient phase there is no effect of DTAB addition on the kinetic parameters at concentrations below the critical micelle concentration (CMC) of the surfactant, super-activity is observed when the acyl–enzyme intermediate reaches the steady state condition. This difference implies that the surfactant does not modify either the formation or the decomposition of the enzyme–substrate complex (transient phase) but notably increases the rate of disruption of the acyl–enzyme intermediate.

Keywords: Dodecyltrimethylammonium bromide; 2-Naphthyl acetate; α-Chymotrypsin

1. Introduction

α-Chymotrypsin (α-CT) is a water-soluble enzyme that catalyzes the hydrolysis of peptidic bonds in proteins, being also able to act both upon amide [1–5] and ester linkages [6]. The activity of the enzyme is modified by the presence of lipid/water interfaces and extensive studies have been reported regarding the characteristics of esters (such as 2-naphthylacetate) and amides (*N*-glutaryl-L-phenylalanine *p*-nitroanilide) hydrolysis in reverse micellar solutions [1,6–9] and aqueous surfactant solutions [2–5]. An overlooked effect regarding the kinetic behaviour of α-CT catalyzed hydrolysis of 2-naphthylacetate (2-NA) in aqueous solutions of surfactants is related with the change of mechanism that can take place when passing from the transient ("burst") to the steady state phases [10].

In previous works [9,11], we have shown that the effect of dodecyltrilmethylammonium bromide (DTAB) upon the catalytic activity of α-CT depends upon the employed substrate: 2-naphthylacetate (2-NA) [9] or *N*-glutaryl-L-phenylalanine *p*-nitroanilide (GPNA) [11]. In particular, at concentrations below the critical micelle concentration (CMC), the presence of the surfactant does not affect the rate of 2-NA hydrolysis [9] but produces a noticeable increase (up to a factor 13) on the rate of hydrolysis of (GPNA) [11]. A tentative explanation of this difference was advanced in terms of the mechanism of α-CT catalysis, represented by the following steps [11–13],



(1)

where S is the substrate, E is the enzyme, (SE) is the enzyme–substrate complex, S'E is the acyl–enzyme intermediate and P_1 and P_2 are the basic and acidic products, respectively.

In particular, it was proposed that the reported differences were not due to the differences in the substrate characteristics but to the different stages at which the reaction rates were measured [11]. The data obtained for GPNA were obtained under conditions where both intermediates (SE and S'E) have

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doi:10.1016/j.molcatb.2004.07.009

reached their steady state concentrations; while the 2-NA data corresponded to "initial" rates and were then obtained prior to attain the steady state concentration of the S'E intermediate. This proposal implies that all the effect of the surfactant is due to changes in k_3 and hence must be observed when the rate of the process is influenced by (S'E) decomposition. In order to test this hypothesis, we have measured the effect of the surfactant upon the rate of 2- NA hydrolysis at times long enough to assure attainment of the steady state condition for the (S'E) intermediate.

2. Experimental section

2.1. Materials

Dodecyltrimethylammonium bromide and α-chymotrysin (Type II, from bovine pancreas, p*I* = 8.8, Sigma) were used as received. Ultrapure water obtained from a Modulab Type II equipment was employed to prepare all the solutions. Tris–(hydroxymethyl) aminomethane (Tris) was a product from Aldrich.

2.2. Measurement of the rate of 2-NA hydrolysis catalyzed by α-CT

The rate of 2-NA hydrolysis, catalyzed by α-CT, was measured at 25 °C in an aqueous buffered solution and in DTAB solutions at pH = 7 (10 mM Tris–HCl buffer) using a Hewlett Packard UV-visible 8453 spectrophotometer equipped with a thermostated cell (3 mL of volume and 1 cm path length) controlled at ±0.1 °C. Because the isoelectric point of the enzyme is 8.8, it has a positive charge under the conditions employed in this work. The reaction was followed by recording the absorbance of 2-naphthol (2-N) at 330 nm ($\varepsilon = 1.53 \times 10^3\ M^{-1}\ cm^{-1}$, either in pure buffer or in the presence of the surfactant) as a function of time. The reaction was initiated by addition of 8 μL of α-CT stock solution (40 mg/mL) to 2.6 mL of a solution containing the desired concentration of 2-NA in buffer (or buffer plus DTAB). Enzyme and substrate solutions were always freshly prepared immediately before their use. Under the first ca. 2 min of reaction the 2-N versus time plots were curved downward. Reported rate values, *V*, correspond to those obtained after a linear 2-N concentration versus time dependence was attained.

3. Results and discussion

Fig. 1 shows a typical plot of the time course of 2-N formation in the hydrolysis of 2-NA catalyzed by α-CT. The data show a high initial rate that readily decays to a steady situation. A similar behavior was observed under all the experimental conditions employed. The transient phase can be ascribed to the time required to reach the steady state condition for the (S'E) intermediate. The rates measured under these last conditions are plotted, as a function of the initial 2-NA concentration, in Fig. 2. The plateau observed at high 2-NA concentrations is in agreement with the Michaelis constant, K_M, value reported for this system in the presence of DTAB below its critical micelle concentration (9). The data of Fig. 2 show that the presence of DTAB, at concentrations below the surfactant CMC (14 mM) [14] increases the rate of the process over all the substrate concentration range considered. In this sense, the data obtained employing 2-NA as substrate follows the same pattern than that obtained employing GPNA [11].

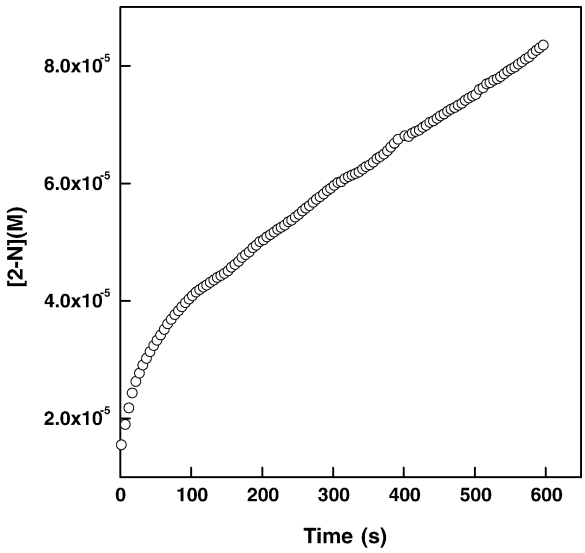

Fig. 1. Time course of 2-N formation in the hydrolysis of 2-NA catalyzed by α-CT. Conditions: 2-NA = 0.15 mM; DTAB = 5 mM.

A simple steady state treatment applied to the intermediates in Eq. (1) gives that, at high 2-NA concentrations, the

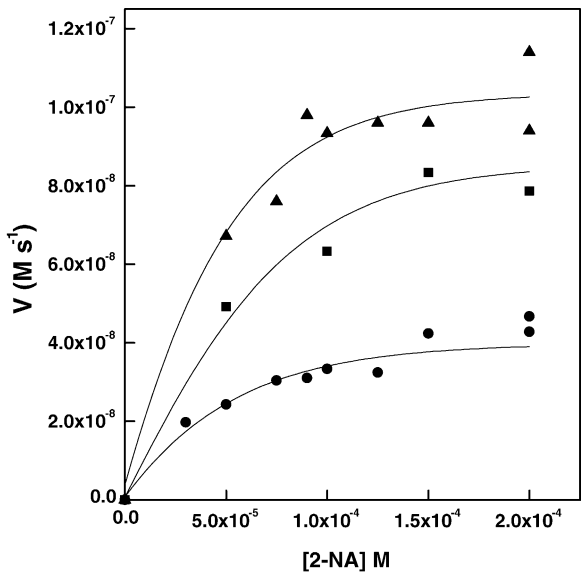

Fig. 2. Effect of DTAB concentration on the relationship between the reaction rate, *V*, and the concentration of 2-NA. (●) In the absence of DTAB (■) DTAB = 5 mM (▲) DTAB = 10 mM.

Table 1
Values of k_3 determined from Eq. (3)

Surfactant concentration (mM)	k_3 (s^{-1})
0	0.0012
5	0.025
10	0.032

rate of the process, V, is given by

$$V = \frac{k_2 k_3 [E]_0}{k_2 + k_3} \quad (2)$$

leading to

$$\frac{1}{k_3} = \frac{[E]_0}{V} - \frac{1}{k_2} \quad (3)$$

Limiting values of $[E]_0/V$ were obtained from the ordinates of double reciprocal plots ($[E]_0/V$ versus $[2\text{-NA}]^{-1}$) of the data shown in Fig. 2. These plots were fairly linear and, in spite of the rather high conversions reached, the extrapolation procedure can be consider to render the correct values of $[E]_0/V$ under substrate saturation conditions. In a previous work [9], we have shown that at below the critical micellar concentration (CMC) of DTAB, the initial rate of 2-NA catalyzed hydrolysis is nearly independent of the surfactant concentration, irrespective of the substrate concentration. This implies that k_2, that can be equated to the catalytic rate constant, k_{cat}, measured at "zero" reaction time, is independent of the surfactant concentration below the CMC. The value derived from the data of reference [9] is $k_2 = 0.124\ s^{-1}$ [15]. Eq (3) allows then to obtain k_3 values as a function of the surfactant concentration. The data obtained are collected in Table 1. These data show that k_3 notably increases in the presence of the surfactant. This effect could be associated to subtle changes in the enzyme conformation resulting from its interaction with DTAB, even below the surfactant CMC. Interestingly, these changes only significantly increase the rate of disruption of the S'E intermediate, without significant changes in the formation of the (SE) complex and/or the leaving rate of the naphtoate group from the initial substrate–enzyme complex.

In conclusion, the effect of DTAB on the behavior of α-CT catalyzed hydrolysis of 2-NA is similar to that shown for the hydrolysis of GPNA when the kinetic data are analyzed under steady state conditions for the corresponding intermediates. For 2-NA hydrolysis, a distinct effect of DTAB is presented when the kinetic data are taken within the transient phase ("burst") or steady state conditions for the acyl–enzyme intermediate.

Acknowledgments

Thanks are given to Dicyt (USACH) and Fondecyt (Project # 1010148) for financial support.

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