

UNUSUAL BEHAVIOUR OF A HYDROPHOBIC BENZIMIDAZOLEMETHANETHIOL IN MICELLAR ESTEROLYSIS

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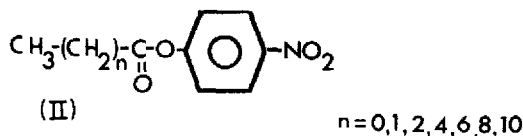
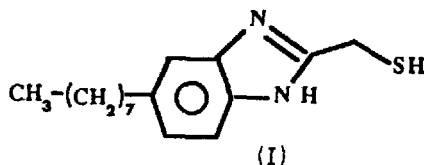
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Abstract

Description of the unusual behaviour of the first term of a new series of straight-chain heterocyclic thiols (benzimidazolemethanethiols) in a micellar system, in the hydrolysis reaction of p-nitrophenylesters.

Cysteine proteases form a large class of enzymes, and their active site is known to contain both a cysteine thiol group and an imidazole heterocycle borne by a histidine residue. In the field of enzyme model reactions, we have reported ^(1,2,3) that small molecules, i.e. benzimidazolemethanethiols, exhibited high esterolytic activity towards p-nitrophenylacetate. In order to modelize the cysteine-enzyme activity more precisely, with the aim of increasing that of the models as a result of hydrophobic effect contribution, we have investigated the esterolytic properties in micellar system of new straight-chain benzimidazole methanethiols towards p-nitrophenyl esters substituted in the same way.

In this paper, we report our first observations concerning the activity of the C₈ thiol (I) (C₈ Bim SH) model towards the hydrolysis of p-nitrophenyl esters of increasing hydrophobic character (C₂, C₃, C₄, C₆, C₈, C₁₀, C₁₂) (II).



The experimental procedure is as follows : the micelle-promoted hydrolysis of the substrates (5×10^{-5} M) with the thiol (2×10^{-4} M) in the cationic surfactant (CTABr concentration = 2.5×10^{-3} M, c.m.c. = 5.10^{-4} M, determined spectrophotometrically by the dye incorporation method at 30° C in HEPES solution) (^{4,5,6,7,8}) was carried out at 30° C (pH = 7.65) in a HEPES buffer (0.05 M) containing NaCl (I = 0.1) in 1 % (v/v) alcohol-water. All solutions were carefully deoxygenated by bubbling Ar, and stock solutions (containing both nucleophile and surfactant in buffer) were prepared by sonication for 1 mn in a water-bath type sonicator (power level 60 W). The reaction was followed by the spectrophotometric determination of the p-nitrophenolate ion (400 nm).

The reactions for the different substrates were observed until total completion, and in all cases exhibited excellent pseudo-first order rate law for more than 90 %. When the runs were performed at ten-minute intervals after preparation of the solutions, hydrolysis reactions were very rapid, with reproducible rate-constant values (Table 1(a)). When the micellar stock solution was left to stand (over 10 mn) before injection of the ester (carefully avoiding oxidation) the kinetic runs showed an important decrease of the esterolysis rate (100 times lower : Table 1(b)). In this case, the reaction also obeys a usual pseudo-first rate law for more than 90 % completion. Although the reaction is slow, it remains twice as fast as the hydrolysis reaction of p-nitrophenylacetate with the non-substituted benzimidazole-methanethiol in 10 % (v/v) alcohol-water medium (buffer phosphates), as is shown by comparison with the second apparent rate constants :

$$k'_2 = \frac{k_{\text{obs}} - k_0}{[\text{thiol}]_{\text{total}}} \quad (k_0 = \text{rate constant due to buffer, } k'_2 = 2 \cdot \text{M}^{-1} \text{ s}^{-1})$$

Table 1. Global rate-constant k_{obs} for the hydrolysis of the different p-nitrophenyl esters [CTABr] = 2.5×10^{-3} M, thiol = C_8 Bim SH (2×10^{-4} M), [Ester] = 5×10^{-5} M, T = 30° C

a. Rapid process

Esters	C ₂	C ₃	C ₄	C ₆	C ₈	C ₁₀	C ₁₂
$10^2 k_{\text{obs}}$ (s ⁻¹)	4.1	6.9	6.9	8.9	11.85	10.8	3.85

b. Slow process

Esters	C ₂	C ₃	C ₄	C ₆	C ₈	C ₁₀	C ₁₂
$10^4 k_{\text{obs}}$ (s ⁻¹)	2.9	3.5	3.6	6.9	8.1	8.1	8.7

This peculiarity is observed whatever the chain-length of the ester. We are confronted with a system which acts according to two processes. A rapid reaction, noted when performing runs for a short while after sonication of the stock solution, and a second slower one which occurs when the solution has been left to stand. This peculiarity is non-reversible. After new sonication of the stock solution, the rapid process is not obtained a second time. A more detailed study of the evolution of this peculiarity has been undertaken with the system C_8 BimSH/ C_{10} ester. The following graph (Figure 1) shows the variations of the kinetic curves profile : the transition from the rapid process to the slow one. The intermediate curve can be decomposed into two distinct parts. The first part, in which the rapid process is dominant, and the second one, which is characteristic of a "purely" slow process.

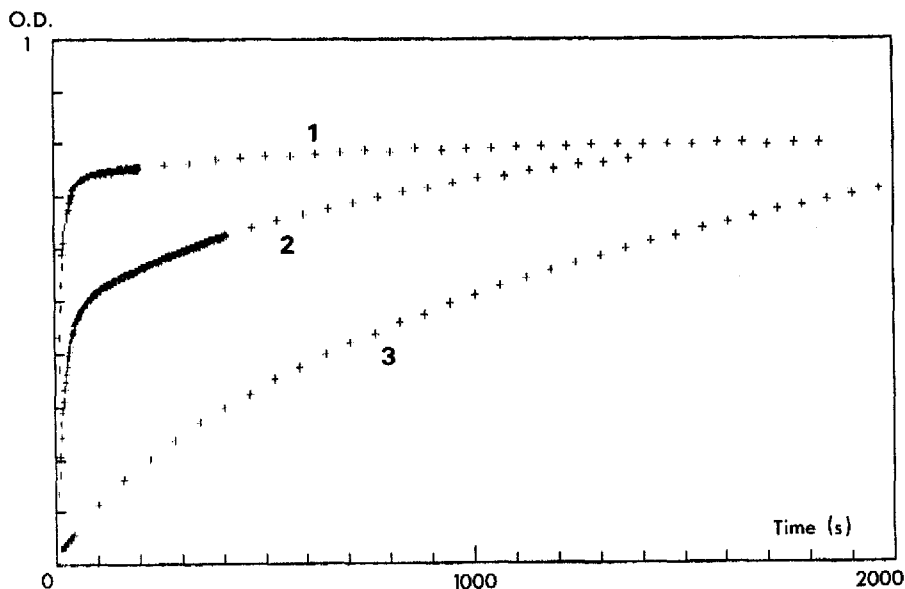


Figure 1. Time-dependent kinetic curves profile in the esterolysis reaction of C_{10} ester with C_8 Bim SH. Conditions identical to those mentioned above.

- 1 t = 1 mn (after sonication)
- 2 t = 30 mn
- 3 t = 3 hrs

A comparative kinetic study performed with an aliphatic thiol (system C_{12} SH/ C_8 ester) (⁹) does not exhibit this feature, even if the solution has been left to stand for a long time (26 hrs). A rate constant similar to that of a rapid process is obtained ($k_{obs} = 9.7 \times 10^{-3} s^{-1}$). The kinetic study of an S-methyl benzimidazole model (2-methylmercapto-methyl-5(6)dodecylbenzimidazole, C_{12} Bim S- CH_3) towards C_{10} ester shows that it undergoes a single process (unchanged kinetic after the solution has been left to stand for 30 hrs $k_{obs} = 1.8 \times 10^{-4} s^{-1}$). Nevertheless, this constant remains 5 times smaller than those corresponding to the slow process for the system C_8 Bim SH/ C_{10} ester.

This phenomenon, which seems peculiar to the benzimidazolemethanethiols (associating the

benzimidazole cycle with the thiol function), is linked to the very structure of this heterocyclic thiol. It is a fact that the important decrease in the reactivity of C₈ Bim SH in micelles cannot be attributed to a modification of the thiol group ; the potentiometric titration of the latter (3 hrs after preparation of the solution : [C₈ Bim SH] = 5 x 10⁻³ M, (CTAB) = 5 x 10⁻² M, (NaCl) = 0,1 M, 1 % alcohol) confirms that it is still present in the same proportions (precision 2 %).

Another important observation is the value at infinite time of the optical density in the two cases (same conditions, pH = 7.65 [Ester] = 5 x 10⁻⁵ M). In the case of the slow process, the final optical density, obtained after complete hydrolysis of the ester, is equal to 0.84. For the rapid process, however, it is equal to 0.79, which corresponds to the normal value obtained for the determination of the molar extinction coefficient of p-nitrophenol. This result shows that there is a particular state which exacerbates the final O.D. (molar extinction coefficient) in the case of the slow process. No definite explanation can yet be put forward, and a more complete study is in progress, using different esters and thiols and considering the influence of the concentration and the nature of the surfactant.

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