Facile Acyl Transfer from Imidazole to the Hydroxy-group in a Cationic Micelle in the Hydrolysis of *p*-Nitrophenyl Acetate

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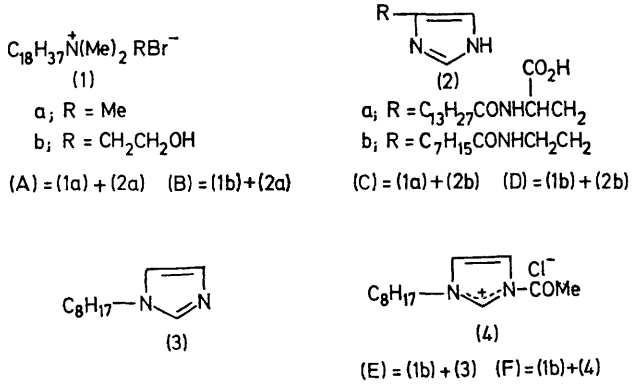
Summary The hydrolysis of *p*-nitrophenyl acetate in the presence of a mixed micelle of *NN*-dimethyl-*N*-2-hydroxyethylstearyl ammonium bromide (**1b**) and 4-imidazolyl derivatives (**2**) has been found to occur by a fast acylation of the imidazole followed by a fast quantitative transfer of the acyl group to the hydroxy-group.

α-CHYMOTRYPSIN catalysed hydrolysis of a non-specific substrate, *e.g.* *p*-nitrophenyl acetate, (PNPA) has been suggested to occur by initial acylation of the active site of the imidazole followed by acyl transfer to the serine hydroxy group.[1] The related non-enzymic model reaction, hydrolysis of PNPA, is known to be catalysed by the micelles of cationic surfactants which have functional groups such as imidazole[2] and 2-hydroxyethyl.[2d,3] It is also known that these groups act as nucleophiles giving acylated products, and that the imidazole is far more reactive than the hydroxy-group. Thus, if these two functional groups coexist, one may expect an acyl group transfer reaction from the imidazole to the hydroxy-group as in the above enzyme reaction. This possibility has now been examined.



The hydrolysis of PNPA was studied using the cationic micellar systems (**1a**), (**1b**), and (A)—(F).† Some examples of rate constants for the formation of *p*-nitrophenol are: $k_{obs} \times 10^5/s^{-1}$ (catalyst); 0·794 (none), 7·94 (**1a**), 22·4 (**1b**), 304 (A), and 377 (B), with [PNPA] = 5×10^{-5}M, [(**1a**)] = [(**1b**)] = 2×10^{-3}M, [(**2a**)] = 2×10^{-4}M, pH 7·10 (0·05 M phosphate buffer), and 30 °C. These rate data appear to parallel those reported previously.[2,3] It should be noted that the rates for (A) and (B) are essentially the same and are mainly determined by the reactivity of the imidazole group. While a sharp difference between (A) and (B) is observed in the deacylation step as shown in the Figure, with both (A) and (B), the acylation of the imidazole is fast with comparable rates. The acylimidazole intermediate is very stable with (A) as catalyst, whereas with (B) it decomposes rapidly. In (B) the acyl group may possibly be transferred to the hydroxy-group of (**1b**). In order to confirm this possibility, (B) was first treated with PNPA until the formation of *p*-nitrophenol at pH 7 and 30 °C was completed. The reaction mixture was then treated with a large excess of hydroxylamine (0·1 M, pH 7, 30 °C) and the time-dependent formation of acetohydroxamic acid was followed colorimetrically to infinity.‡ Three runs with the concentration of (B) constant ([**1b**] = 2×10^{-3}M + [**2a**] = 2×10^{-4}M) and that of PNPA being varied (3, 5, and 10×10^{-4}M) gave three values of absorbance differences ($A_\infty - A_0 = 0{\cdot}307$,

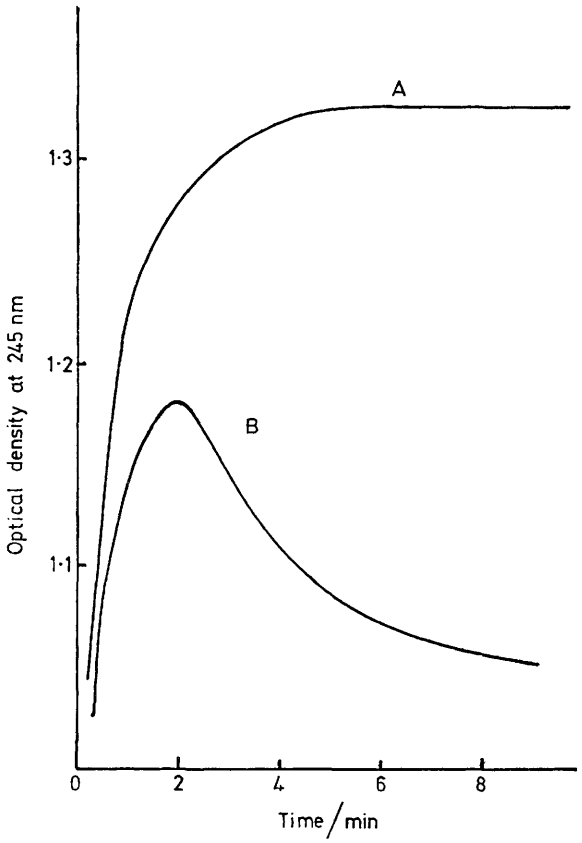


FIGURE Formation and decomposition of the acylimidazole intermediate in the hydrolysis of PNPA: (A) = (**1a**) (5×10^{-2} M) + (**2a**) (5×10^{-3} M), (B) = (**1b**) (5×10^{-2} M) + (**2a**) (5×10^{-3}M), [PNPA] = $1{\cdot}6 \times 10^{-4}$ M, pH 7·10 (0·05 M phosphate buffer), 25 °C.

† (**1**), (**2**), (**3**), and (**4**) are known compounds (see refs. 2 and 3).

‡ Based on the absorption of acetohydroxamic acid–$FeCl_3$ complex (510 nm, $\epsilon = 1050$); for the method see L.-H. King and E. T. Kaiser, *J. Amer. Chem. Soc.*, 1974, **96**, 1410.

0·550, and 1·05 at 510 nm) which corresponded to quantitative detection of the acetyl group of PNPA.§

From the above findings it may be concluded that in the presence of (**2a**) the acylation of (**1b**) occurs exclusively through acyl-transfer from the imidazole, since it is much more reactive than the hydroxy-group towards PNPA. Similar results for the fate of the acetyl group have also been obtained for (C) and (D), where (**2b**) has no anionic group (*e.g.* CO_2^- group in **2a**) with which to form an ion-pair with the cationic species (**1a**) and (**1b**). Thus an efficient acyl-transfer in (B) and (D) appears to be due simply to the enhanced nucleophilic reactivity of (**1b**) under the micellar conditions and at neutral pH. It is interesting that such an acyl-transfer was not detected in the case of system (E) in spite of an enhanced rate of formation of *p*-nitrophenol in the presence of (**3**).¶ Acyl-transfer was not observed when (**4**) was mixed with (**1b**) (*e.g.* system F). This may be consistent with the observation that the rate of decomposition of (**4**) (*e.g.* hydrolysis) is not affected by cationic surfactants, but is strongly inhibited by an anionic surfactant.**

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§ The following mixtures were also treated with hydroxylamine as control experiments: (a) the acetate of (**1b**), (b) the acetate of (**1b**) + (**2a**) + *p*-nitrophenol, and (c) (**1b**) + (**2a**) + free acetate + *p*-nitrophenol. A quantitative yield of acetohydroxamic acid was obtained from (a) and (b), while none was obtained from (c).

¶ Detection of the acylimidazole intermediate (at 245 nm), however, was unsuccessful.

** The rate of hydrolysis of (**4**), $k_{obs} = 0{\cdot}739\ s^{-1}$ [concentration = $6{\cdot}5 \times 10^{-4}$M, 20 °C, and pH 7·21 (0·05 M phosphate buffer)] was unchanged in the presence of (**1a**) and (**1b**) ($\leqslant 4 \times 10^{-3}$ M), but decreased to $0{\cdot}0617\ s^{-1}$ in the presence of sodium lauryl sulphate ($2{\cdot}01 \times 10^{-3}$M).

[1] J. F. Kirsch and C. D. Hubbard, *Biochemistry*, 1972, **11**, 2483. For a specific substrate, the imidazole is known to act as a general acid and base and the initial acylation is on the serine hydroxy-group: 'The Enzymes', Vol. 3, 3rd edn., Ed. P. D. Boyer, 1971, 1911.

[2] (a) Review: J. H. Fendler and E. J. Fendler, 'Catalysis in Micellar and Macromolecular Systems,' Academic Press, New York, 1975; (b) C. Gitler and A. Ochoa-solano, *J. Amer. Chem. Soc.*, 1968, **90**, 5004; (c) W. Tagaki, M. Chigira, T. Amada, and Y. Yano, *J.C.S. Chem. Comm.*, 1972, 219; (d) R. A. Moss, R. C. Nahas, S. Ramaswami, and W. J. Sanders, *Tetrahedron Letters*, 1975, 3379, and related refs. therein.

[3] K. Martinek, A. V. Levashor, and I. V. Berezin, *Tetrahedron Letters*, 1975, 1215, and related refs. therein.