The Carboxypeptidase Y-catalysed Hydrolysis of Aryl Trimethylacetates: the Nature of the Deacylation Reaction of Trimethylacetylcarboxypeptidase Y

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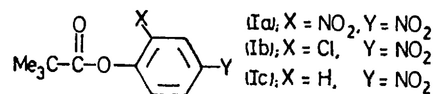
Summary The identical k_{cat} values obtained for the trimethylacetyl esters of 4-nitrophenol, 2,4-dinitrophenol, and 2-chloro-4-nitrophenol support the intermediacy of a trimethylacetyl enzyme in the esterase action of yeast carboxypeptidase Y, the deacylation of which depends on the ionization of an acidic species ($pK_{app} = 5{\cdot}1$) with limiting k_{cat} values of $0{\cdot}9 \times 10^{-2}\,s^{-1}$ and $2{\cdot}2 \times 10^{-2}\,s^{-1}$ in acidic and basic solutions, respectively.

In contrast to the activity of the pancreatic enzyme carboxypeptidase A, that of carboxypeptidase Y, a *C*-terminal exopeptidase found in yeast,[1] apparently has no metal ion requirement. A serine residue and, possibly, a cysteine residue may be crucial active-site constituents in carboxypeptidase Y.[1b,2] This enzyme has a broad specificity and could be very important in protein chemistry if its use in protein sequence analysis continues.

Under substrate in excess conditions the reaction of 4-nitrophenyl trimethylacetate with carboxypeptidase Y yields the 4-nitrophenolate anion in a rapid pseudo-first order stage, followed by a slower zero-order turnover process.[3] It has been suggested that an acyl-enzyme is formed in the rapid step and that the slower stage involves its rate-controlling decomposition. We now report studies on additional trimethylacetyl esters which agree with this hypothesis.



The carboxypeptidase Y samples used in this work were prepared by the method of Hayashi *et al.*[2c] and had a molecular weight of 61,000 by SDS gel electrophoresis.[4] Under conditions in which the initial substrate concentration was considerably greater than that of the enzyme, the reactions of the 2,4-dinitrophenyl ester, (Ia), and the

2-chloro-4-nitrophenyl ester, (Ib), with carboxypeptidase Y exhibited 'burst' kinetics with respect to phenolate ion release. After correction for spontaneous and turnover hydrolysis of the substrate,[5] the 'burst' regions could be analysed in terms of pseudo first-order kinetics, but it is the subsequent zero-order region from which k_{cat} can be evaluated ($k_{cat}[E]_0$ = observed zero-order rate constant). Values of k_{cat} measured at pH 7·35 are shown in the Table.

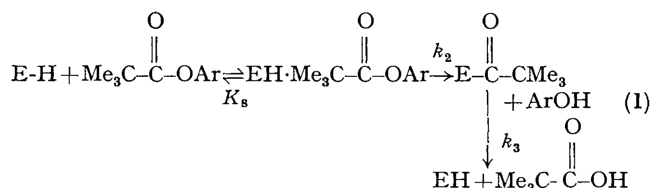
TABLE

Values of k_{cat} for the reactions of aryl trimethylacetates (I) with carboxypeptidase Y[a]

Compound	pK of product phenol	k_{cat}/s^{-1}
(Ia)	3·96[b]	$(2·72 \pm 0·10) \times 10^{-2}$
(Ib)	5·45[c]	$(2·72 \pm 0·10) \times 10^{-2}$
(Ic)	7·04[d]	$(2·92 \pm 0·07) \times 10^{-2}$

[a] Measured in pH 7·35 phosphate buffer (ionic strength 0·1) at 25° in the presence of 3·2% (*v/v*) acetonitrile. [b] 'CRC Handbook of Chemistry and Physics,' 50th edn., 1969, table D120. [c] V. E. Bower and R. A. Robinson, *J. Phys. Chem.*, 1960, **64**, 1078. [d] F. J. Kézdy and M. L. Bender, *Biochemistry*, 1962, **1**, 1097.

The close agreement between the values of k_{cat} for the carboxypeptidase Y-catalysed hydrolysis of esters (Ia)—(Ic) is readily interpreted in terms of a common rate determining step for these reactions, the breakdown of trimethylacetyl carboxypeptidase Y, (step k_3 in equation 1). The steady-state treatment of equation 1[5c] leads to equation 2. For (Ia)—(Ic), $k_2 >> k_3$. If the pK_a values of the conjugate acids of the leaving groups listed in the Table are examined, it can be seen that the fortuitous identity of the k_{cat} values is unlikely.†



$$k_{cat} = \frac{k_2 k_3}{k_2 + k_3} \text{ and } K_{m_{app}} = K_S \cdot \frac{k_3}{k_2 + k_3} \quad (2)$$

For (Ia) in acidic solution (pH 3) the limiting k_{cat} value is $0·9 \times 10^{-2}\ s^{-1}$ and in alkaline solution (pH 10) is $2·2 \times 10^{-2}\ s^{-1}$ with a dependence of this kinetic parameter on the ionization of an acidic group of pK_{app} = 5·1.‡ This low sensitivity of k_{cat} to changes in pH contrasts markedly with the behaviour seen in the deacylation reactions of serine proteinases such as α-chymotrypsin[6] It is, however similar to measurements on the pH dependency of k_{cat}, values for the reaction of carboxypeptidase A with some esters such as *O*-hippuryl-L-β-phenyllactate,[7] which may be significant in view of the proximity of a glutamate residue to the active site serine in carboxypeptidase Y.[2c] Further studies to establish whether intermediates similar to those seen in the nitrophenyl esterase action of the yeast enzyme are involved in the exopeptidase action and, to define the active site more clearly are in progress.

We thank the National Institute of Arthritis, Metabolic and Digestive Diseases for support.

(*Received*, 18*th March* 1975; *Com.* 327.)

† One indication of the influence of the leaving group on reactivity is a β value of −0·7 for the reactions of the aryl trimethyl-acetates with imidazole.

‡ A different batch of carboxypeptidase Y was used in this pH rate study than was employed to obtain the data shown in the Table. We are working on an active site titration procedure which should allow us to put kinetic results measured with batches of enzyme with differing activities on the same scale.

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