Enantiomeric Specificity in the Cyclohexa-amylose-catalysed Hydrolysis of 3-Carboxy-2,2,5,5-tetramethylpyrrolidin-1-oxy *m*-Nitrophenyl Ester

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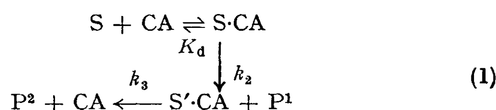
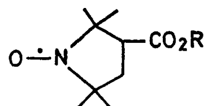
Summary The asymmetric catalytic effect in the hydrolysis of racemic 3-carboxy-2,2,5,5-tetramethylpyrrolidin-1-oxy *m*-nitrophenyl ester by cyclohexa-amylose is the largest found so far in a model-enzyme catalysed hydrolysis of a carboxylic acid ester.

WE report here a very high enantiomeric specificity in the reaction of the toroidal polysaccharide cyclohexa-amylose with racemic 3-carboxy-2,2,5,5-tetramethylpyrrolidin-1-oxy *m*-nitrophenyl ester (I), a substrate containing an asymmetric carbon atom adjacent to the carbonyl group of the hydrolytically labile ester function.

There is ample evidence[1,2] that hydrolyses of phenyl esters catalysed by cycloamyloses follow the route of equation (1) which is similar to catalyses by the serine proteinase, α-chymotrypsin.[3]†



$$\mathrm{S} + \mathrm{CA} \underset{K_d}{\rightleftharpoons} \mathrm{S{\cdot}CA} \xrightarrow{k_2} \mathrm{S'{\cdot}CA} + \mathrm{P}^1;\quad \mathrm{S'{\cdot}CA} \xrightarrow{k_3} \mathrm{P}^2 + \mathrm{CA} \qquad (1)$$



(I) R = m-$NO_2{\cdot}C_6H_4$
(II) R = -H

Measurements at 392 nm at pH 8·62 and 25·0° on the rate of production of *m*-nitrophenolate from $1{\cdot}02 \times 10^{-4}$ M-(±)-(I) in the presence of $1{\cdot}48 \times 10^{-2}$M-cyclohexa-amylose indicate the process catalysed by the cyclodextrin to be biphasic. The rate data can be analysed in terms of a fast and a slow reaction both following pseudo-first-order rate laws and accounting, respectively, for 50% of the decomposition of (I). Measurements on the reaction of (±)-(I) with varying concentrations of cyclohexa-amylose gave values of $K_d = 0{\cdot}013 \pm 0{\cdot}002$ M and $k_2 = 0{\cdot}022 \pm 0{\cdot}002\ \mathrm{s}^{-1}$ for the fast reaction and $K_d = 0{\cdot}013 \pm 0{\cdot}005$ M and $k_2 = 0{\cdot}0032 \pm 0{\cdot}0006\ \mathrm{s}^{-1}$ for the slow reaction.

We believe that the biphasic character of the reaction of (±)-(I) with cyclohexa-amylose is due to differences in the reactivity of the two enantiomers of (I) with the catalyst. To test this, we obtained (+)-(II) from (±)-(II) by resolution with brucine and converted (+)-(II) into (+)-(I), m.p. 46—49°, $[\alpha]_D^{25} + 29 \pm 3°$ (MeCN). In contrast to the reaction of (±)-(I), (+)-(I) reacts monophasically with excess of cyclohexa-amylose. Values of $K_d = 0{\cdot}019 \pm 0{\cdot}002$ and $k_2 = 0{\cdot}025 \pm 0{\cdot}002\ \mathrm{s}^{-1}$ were calculated for the reaction of (+)-(I) at pH 8·62 (Tris–HCl buffer) and 25.0°, agreeing well with the findings for the fast reaction with (±)-(I). We conclude the higher reactivity of cyclohexa-amylose with (+)-(I) compared with (—)-(I) to be responsible for the biphasic character of its reaction with (I). Further evidence was obtained from a partial hydrolysis of racemic-(I) by cyclohexa-amylose; the unchanged ester was the expected partially resolved (−)-(I) species.

By a procedure described earlier,[4] the inclusion of "Michaelis" complexes of (+)-(I) and (±)-(I) with cyclohexa-amylose in acidic solution were detected by e.s.r. Although dissociation constants could not be calculated accurately from our e.s.r. measurements, the K_d values thus estimated at pH 4·99 (acetate buffer) for both (+)-(I) and (±)-(I) were similar to those computed from kinetic measurements at pH 8·62. No qualitative differences were seen in the e.s.r. spectra of the cyclohexa-amylose complexes of (±)-(I) and (+)-(I).

E.s.r. spectra of acylated cyclohexa-amyloses prepared from both (+)-(I) and (±)-(I)[2,4] agreed within experimental error, and e.s.r. measurements of the rate constants, k_3, for their deacylation at pH 9·74 (carbonate buffer) gave identical values of $1{\cdot}1 \times 10^{-4}\ \mathrm{s}^{-1}$.

In the reaction of ester (I) with cyclohexa-amylose, the enantiomeric specificity seen thus arises only because the rate constants, k_2, differ for the (+)- and (−)-isomers of the substrate. In contrast, no appreciable enantiomeric specificity had been observed for any steps in which (±)-(I) was hydrolysed by cyclohepta-amylose.[4] Although cyclohepta-amylose binds (±)-(I) about twenty times more strongly than does cyclohexa-amylose, the acylation step proceeding from the "Michaelis" complex occurs seventy times faster with (+)-(I) and nine times faster with (−)-(I) in the case of cyclohexa-amylose.‡ These differences

† In equation (1), S represents the ester, CA the cycloamylose, S·CA the inclusion or "Michaelis" complex, S′·CA the acylcycloamylose, P[1] the product alcohol, and P[2] the product acid.

‡ Numbers comparing cyclohepta-amylose catalysis have been revised from ref. 4. (R. M. Paton.)

illustrate the ability of the cycloamyloses to show significant "enzymatic" specificity for both acyl and phenolic moieties of phenyl esters.

Thus, to the list of similarities between cycloamylose- and enzyme-catalysed hydrolyses of carboxylic acid derivatives can now be added enantiomeric specificity. In both systems the stereospecificity observed is much larger for acylation than for deacylation[5] and reinforces the analogy between enzymatic and model reactions. While the enantiomeric specificity described here is substantially less than that of chymotrypsin for substrates such as *N*-acetyl-L- and *N*-acetyl-D-tryptophanamide,[6] the asymmetric catalytic effect observed is to our knowledge the largest so far found in a model-enzyme carboxylic esterase.§

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§ The asymmetric effects on cyclohexa-amylose-catalysed hydrolysis of some substituted ethyl mandelates[7] were far smaller. Van Hooidonk[8] reported recently that cyclohexa-amylose can show high enantiomeric specificity against asymmetric centres involving phosphorus.

[1] R. L. Van Etten, J. F. Sebastian, G. A. Clowes, and M. L. Bender, *J. Amer. Chem. Soc.*, 1967, **89**, 3242.
[2] R. L. Van Etten, G. A. Clowes, J. F. Sebastian, and M. L. Bender, *J. Amer. Chem. Soc.*, 1967, **89**, 3253.
[3] M. L. Bender and F. J. Kézdy, *Ann. Rev. Biochem.*, 1965, **34**, 49.
[4] R. M. Paton and E. T. Kaiser, *J. Amer. Chem. Soc.*, 1970, **92**, 4723.
[5] M. L. Bender, F. J. Kézdy, and C. R. Gunter, *J. Amer. Chem. Soc.*, 1964, **86**, 3714.
[6] R. J. Foster and C. Niemann, *J. Amer. Chem. Soc.*, 1955, **77**, 1886; R. J. Foster, H. J. Shine and C. Niemann, *ibid.*, p. 2378; D. W. Ingles, J. R. Knowles, and J. A. Tomlinson, *Biochem. Biophys. Res. Comm.*, 1966, **23**, 619.
[7] F. Cramer and W. Dietsche, *Chem. Ber.*, 1959, **92**, 1739.
[8] C. VanHooidonk and J. C. A. E. Breebaart-Hansen, *Rec. Trav. chim.*, 1970, **70**, 289; C. Van Hooidonk and C. C. Groos, *ibid.*, p. 845.