Specificity in the Cyclohepta-amylose-catalysed Hydrolysis of Penicillins

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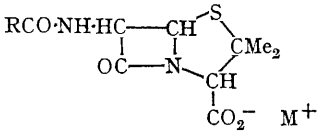
Summary Cyclohepta-amylose (BCD) catalyses the hydrolysis of penicillins (I) *via* the formation of inclusion complexes in which BCD exhibits side-chain specificity.

RECENT interest in the ability of the cycloamyloses (cyclic α-1,4-linked D-glucose polymers containing 6, 7, or 8 glucose residues per molecule) and their derivatives to catalyse a number of chemical reactions[1,2] has led to their description as enzyme models. Their catalytic properties have been described[1] in terms of the formation of cycloamylose–substrate inclusion complexes within the hydrophobic cavity and subsequent catalysis by hydroxy-groups of the sugar. Some degree of stereospecificity has been observed in the cycloamylose-catalysed hydrolysis of substituted phenyl acetates and benzoates,[1] depending on the nature of the fit of the substrate within the cavity and the consequent orientation of the ester carbonyl relative to the catalytic secondary alkoxide ion.

We now describe the cyclohepta-amylose-catalysed hydrolysis of penicillins (I) in which cyclohepta-amylose (BCD)† can be characterized as a simple model for penicillinase[3] (β-lactamase). The rate of β-lactam cleavage of penicillins (I) in the presence (k_{obs}) and absence (k_{hyd}) of BCD was measured under mildly alkaline conditions ($3{\cdot}0 \times 10^{-4}$M-penicillin, pH 10·24, μ = 1·0M, 31·5°, solvent water). In the presence of an *excess* of BCD (0·004—0·020 M) the



Pencillins (I)

M = K or Na
R = side-chain (See Table)

loss of penicillin[4] obeyed an apparent-first-order rate law (often to 93% loss of β-lactam) and the acid liberated in the *overall* reaction corresponded to 100% conversion of penicillin into penicilloic acid, which was determined by (a) pH-stat and (b) a penicilloic acid-specific assay[5] on the reaction mixture at t_∞. The variation of the apparent-first-order rate constants for *loss* of penicillin as a function of BCD concentration in the presence of an *excess* of BCD revealed Michaelis–Menten-like kinetics (see Scheme). Reaction scheme for hydrolysis of penicillins (I) in the

† BCD denotes β-cyclodextrin, another common name, and will be used as an abbreviation of cyclohepta-amylose.

presence of an *excess* of BCD at pH 10·24. Equations (1) and (2) refer to *loss* of penicillin under these conditions, where

SCHEME

$$P + BCD \underset{K(m_{app})}{\rightleftharpoons} P\cdots BCD \xrightarrow{k_2} P\text{–}BCD \xrightarrow{k_3} PA + BCD$$

$$P + OH^- \xrightarrow{k_{hyd}} PA$$

$$\text{Rate} = \frac{k_2(BCD)}{(BCD) + K(m_{app})}(P) + k_{hyd}(P) \quad (1)$$

$$k_{obs} = \frac{k_2(BCD)}{(BCD) + K(m_{app})} + k_{hyd} \quad (2)$$

$$K(m_{app}) = \frac{(P)\,(BCD)}{(P\cdots BCD)}$$

P = penicillin; PA = penicilloic acid; BCD = cyclohepta-amylose; P · · · BCD = inclusion complex; P–BCD = covalent intermediate (penicilloyl-BCD).

Under these conditions the rate and k_{obs} for *loss* of penicillin are given by equations (1) and (2), respectively. Plots of $(k_{obs} - k_{hyd})$ against $(k_{obs} - k_{hyd})/(BCD)$ for each penicillin afforded straight lines from which were evaluated k_2 (maximum rate constant for fully complexed penicillins), $K(m_{app})$, and k_2/k_{hyd} (k_2 relative to the alkaline hydrolysis rate under the same conditions) (see Table for data).

Cyclohepta-amylose was shown to accelerate (20—80 fold) β-lactam cleavage of penicillins when compared (k_2/k_{hyd}) to alkaline hydrolysis under the same conditions.

Variation of the penicillin side-chain revealed considerable specificity in substrate–BCD binding. $K(m_{app})$ (4×10^{-2} to 3×10^{-3} molar) was much lower (corresponding to stronger complexing) than for previously reported penicillinase models[6] and closer to values reported for actual enzyme–penicillin complexes.[7] We believe this to be the first model for penicillinase which exhibits both strong and specific binding of the substrate side-chain. Molecular models of the penicillin–BCD complexes show the penicillin to be quite flexible (the carbonyl carbon of the β-lactam being four atoms removed from the side-chain but accessible to the hydroxy-groups). In this situation the orientation of the labile group in the complex (and hence catalytic specificity) would be affected minimally by stereochemical requirements of hydrophobic binding.

In the presence of either an excess of BCD (apparent-first-order loss of penicillin) or an excess of penicillin (zero-order loss of penicillin at saturation) the rate of loss of penicillin was always *faster* than the rate of formation of final product, penicilloic acid. This result indicates the presence of an intermediate in the reaction pathway. It was possible to demonstrate that this intermediate was a penicilloyl-cycloamylose by using an assay[5] which is specific for penicilloyl derivatives (amides and esters) and penicilloic acid, and able to distinguish between them. For example, in the presence of 0·08M-benzylpenicillin and 0·02M-BCD at pH 10·24, μ = 1·0 M, it was calculated from the experimental rates of penicillin loss and penicilloic acid formation that, after 10 min., the concentration of intermediate was 0·0144 M corresponding to 72% BCD covalently bound to penicillin. Thus, the cycloamyloses and suitably constituted derivatives, which emulate the action of certain hydrolytic enzymes,[1,8] may prove valuable in the elucidation of the mechanism of penicillinase action.

TABLE

Variation of k_2 *and* $K(m_{app})$ (*see Scheme*) *as a function of side-chain for a variety of penicillins* (I) *at* pH 10·24, μ = 1·0 M, 31·5° *in water.*

Side-chain R	k_2 min.$^{-1}$	$10^3 \times k_{hyd}$ min.$^{-1}$	k_2/k_{hyd}	$10^3 \times K(m_{app})$ molar
Me	0·169	4·12	41	37·0
$PhCH_2$	0·326	4·24	77	41·0
Ph_2CH	0·147	4·73	31	3·2
Ph_3C	0·642	15·7	41	3·7
$C_6H_3(OMe_2)$-2,6 ..	0·073	3·53	21	12·0
$Me[CH_2]_8$-	0·18	3·79	47	20·0

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