The Effect of Lysozyme on some Glycosides which contain *N*-Acetylxylosamine Residues

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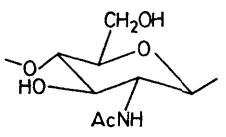
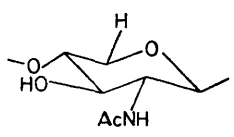
Summary Several aryl glycosides of oligosaccharides containing an *N*-acetylxylosamine residue are shown to be very poor substrates of lysozyme, in accord with Phillips' theory of substrate distortion.

ONE of the central features of the mechanism of action of lysozyme proposed by Phillips and his co-workers is that when a substrate is bound in the productive mode the residue in the D site is distorted into or towards a half-chair conformation owing to non-bonding interactions between the CH_2OH group and the enzyme.[1] This conformation is that expected for an intermediate carbonium ion and hence the expenditure of energy required to achieve a transition state resembling this ion will be reduced and a rate enhancement will result. Two predictions can be made on the basis of this theory: (i) substrates which have the CH_2OH group of the residue, occupying the D site in the productive mode of binding, replaced by H should be hydrolysed much more slowly; (ii) such substrates should be bound more strongly to lysozyme with the modified residue in the D-site.



(I)



(II)

We have tested the first of these predictions by studying the effect of lysozyme on the rate hydrolysis of a series of *p*-nitrophenyl and 3,4-dinitrophenyl glycosides of oligo-

saccharides containing *N*-acetylglucosamine (NAG)† residues and one *N*-acetyl-β-D-xylosamine (NAX)† residue (see Table 1). These were all prepared by the lysozyme-catalysed transglycosylation reactions from *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-xyloside or 3,4-dinitrophenyl 2-acetamido-2-deoxy-β-D-xyloside with NAG-4, NAG-5, or NAG-6. It has previously been shown that *N*-acetylxylosamine forms β-1,4-bonds in these reactions[2] and we have confirmed this by synthesizing NAG–NAX–OPNP from NAG–NAX prepared chemically as described by Chipman[2] when material identical with that obtained enzymically was obtained.

TABLE 1

A comparison of the values of k_{cat}/K_m (l mol^{-1} s^{-1}) at 40° and pH 5·2 of the hydrolyses of some aryl glycosides catalysed by hens' egg-white lysozyme

NAG–NAG–NAX–OPNP	0	NAG–NAG–NAG–OPNP	0·20[a]
NAG–NAG–NAG–NAX–OPNP	<0·002[b]	NAG–NAG–NAG–NAG–OPNP	0·95[a]
NAG–NAX–O–3,4–DNP	<0·004[b]	NAG–NAG–O–3,4–DNP	0·064[c]
NAG–NAG–NAX–O–3,4–DNP	<0·006[b]	NAG–NAG–NAG–O–3,4–DNP	7·9[c]

[a] Ref. 3 at pH 5·08. [b] Upper limit fixed by reproducibility of measurements of the rate of spontaneous hydrolysis. [c] Unpublished observations of F. W. Ballardie, Ph.D. thesis University of Glasgow, 1973.

TABLE 2

A comparison of the dissociation constants (K_I/M) for the complexes of some aryl glycosides with hens' egg-white lysozyme at 40° and pH 5·2[a]

NAG–NAX–OPNP	6×10^{-5}	NAG–NAG–OPNP	5×10^{-4}
NAG–NAX–NAX–OPNP	6×10^{-6}	NAG–NAG–NAG–OPNP	$1{\cdot}6 \times 10^{-5}$

[a] Determined from inhibition of the hydrolysis of 3,4-dinitrophenyl tetra-*N*-acetyl-β-chitotetraoside.

The results given in Table 1 show that replacement of the NAG-residue (I) adjacent to the aryloxy-group by a NAX-residue (II) yields compounds whose hydrolyses are uncatalysed by lysozyme. The "all NAG" substrates in the right hand column show no induction periods and appear to be normal substrates with the hydrolyses occurring initially without transglycosylation. The values of k_{cat}/K_m should therefore be independent of any constants for transglycosylation as well as being independent of the equilibrium constants for non-productive binding. The results for NAG–NAG–NAG–O–3,4-DNP and NAG–NAG–NAX–O–3,4-DNP show a ratio of k_{cat}/K_m of at least 1300, corresponding to a difference in the free energy of activation of at least 4·4 kcal mol^{-1}.‡ Thus if the proposed explanation for the difference in rates of the lysozyme-catalysed reactions is correct this is a lower limit for the contribution to the driving force ascribable to substrate distortion.

Table 2 gives a comparison of the dissociation constants of the enzyme-glycoside complexes for two sets of compounds measured by studying inhibition of the lysozyme catalysed hydrolyses of 3,4-dinitrophenyl tetra-*N*-acetyl-β-chitotetraoside.[3] These results, which are similar to those reported for the free sugars by van Eikeren and Chipman,[4] are less definitive because the measured constant is a macroscopic constant depending on the equilibrium constants for all modes of binding whereas the second prediction made above refers only to binding with a NAG residue in the D-site which is probably a minor mode. The measured variation of these constants on replacing a NAG by a NAX residue could therefore arise from a variation in any one of several equilibrium constants. However the observed stronger binding of the compounds with a NAX residue is not inconsistent with the above prediction.

The results reported above in no way prove that substrate distortion is an important factor in lysozyme-catalysed hydrolyses but merely provide an additional set of experimental results which are those predicted by this theory. There may be other explanations of all the observations but the experiments which demonstrate the low reactivity of the glycosides containing NAX residues have the advantage over previously reported experimental tests[4,5] in that they are independent of any complications arising from non-productive binding.

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† Throughout this communication a 2-acetamido-2-deoxy-D-glucopyranosyl residue which is β-1,4-linked in an oligosaccharide derivative is abbreviated NAG and a 2-acetamido-2-deoxy-D-xylopyranosyl residue similarly linked is abbreviated NAX. *p*-Nitrophenyl is abbreviated PNP and 3,4-dinitrophenyl is abbreviated 3,4-DNP.

‡ Examination of the reaction products by thin-layer chromatography shows that cleavage between NAG residues and between NAG and NAX residues occurs but that the relative rates of these processes cannot account for different rates of release of the phenol from the two series.

[1] See T. Imoto, L. N. Johnson, A. C. T. North, D. C. Phillips, and J. A. Rupley, in 'The Enzymes,' ed. P. D. Boyer, vol. VII, 3rd edn., 1972, pp. 712, 855, 865.
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