Planarity as a Factor in Determining the Rate Constant and Mechanism of Acetal Hydrolysis

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Summary Investigations of the hydrolysis of methyl-α-D-altropyranoside (II), methyl-2,6-anhydro-α-D-altropyranoside (I), and methyl-α-D-glucopyranoside (III) have established that ground-state planarity of the C-2, C-1, O, C-5 region of the glycoside attained in (I) does not lower the energy barrier sufficiently to alter the mechanism from an $A1$ to an S_E2 process (the increase in the rate constant associated with the $A1$ process for the 2,6-anhydroglycopyranoside is much smaller than that established for the 2-deoxyglycopyranosides).

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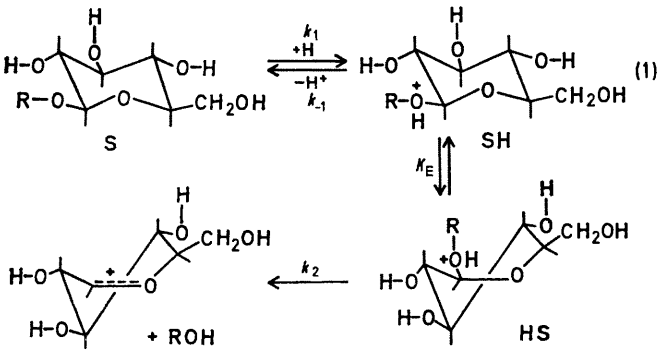
THE distinguishing features of the mechanism of lysozyme action, based on *X*-ray model building,[1] include: (i) involvement of Glu-35 as a general acid catalytic species; (ii) Asp-52 as an electrostatic catalyst; and (iii) substrate constraint, at the expense of binding energy, which provides a coplanar region in the ground state at the locus of incipient oxocarbonium ion formation.[2] Models to assess the efficiency of neighbouring carboxyl groups as general acids and intramolecular electrostatic catalysts have been considered by us.[3] We report our results on one model

designed to ascertain the kinetic and mechanistic significance of constraining the ground-state conformation of a glycoside so that planarity is attained over the C-2, C-1, O, C-5 region.



SCHEME

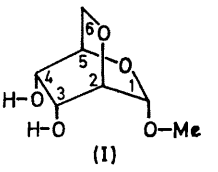
Specific acid-catalysed hydrolysis of glycopyranosides involves pre-equilibrium protonation followed by fission between the exocyclic oxygen bond and the C-1 atom to provide a cyclic oxocarbonium ion which undergoes a rapid nucleophilic attack by water to produce the reducing sugar. This mechanism is based on studies of simple acetal hydrolysis and was suggested for glycoside hydrolysis by Edwards.[4] The Edwards hypothesis incorporates the assumption that the glycoside being hydrolysed attains in the transition state the half-chair conformation which provides planarity in the C-2, C-1, O, C-5 region required for maximum overlap of the non-bonding electrons of the ring oxygen with the developing carbonium ion at C-1. If it were assumed that the rate of solvolysis were proportional to the mole fraction of protonated glycoside in the half-chair conformation (Scheme) then the kinetic Equation (2) would pertain. If K_E is quite large it is conceivable

$$V = k_1k_2K_E[\text{Glycoside}][H_3O^+]/k_{-1} + k_2K_E \qquad (2)$$

that $k_2K_E > k_{-1}$ so that Equation 2 would become Equation 3 and the bond breaking step would be concerted with

$$V = k_1[\text{Glycoside}][N_3O^+] = k_1[\text{Glycoside}][\text{General acid}] \qquad (3)$$

protonation (*i.e.*, the hydrolysis would become general acid catalysed). In the suggested mechanism for lysozyme $K_E = \infty$ allowing the undissociated carboxyl group of Glu-35 to act as a general acid catalyst. If these suppositions are correct then it would be anticipated that for a glycopyranoside whose structure incorporated the planarity of HS, hydrolysis catalysed by H_3O^+ would be facilitated and hydrolysis would be dependent upon the concentration of general acid species in solution. A suitable model to test the kinetic significance of planarity is present in methyl-α-D-2,6-anhydroaltropyranoside (I). A bicyclic system, as present in (I), is found in bicyclo[2,2,2]octane which has been shown by *X*-ray crystallography to be a rigid strain-free system with maximal distortion from complete planarity not greater than 5°.[5] The incipient oxocarbonium ion formed on acid-catalysed hydrolysis of (I) should, therefore, be developed within the planar "cage" structure.



In order to ascertain if (I) were subject to general acid-catalysed hydrolysis, experiments were carried out at constant pH in serial diluted buffers of dichloroacetic acid [$pK_a = 1{\cdot}28$; pH = 1·52 (70°)] and formic acid [$pK_a = 3{\cdot}77$; pH = 3·54 (70°)]. If general acid catalysis were operative then Equation 4 should pertain. Plots of

$$k_{obs} = k_{HA}[HA] + k_Ha_H^+ \qquad (4)$$

$k_{obs}/[HA]$ against $a_H/[HA]$ should yield k_{HA} as intercept and k_H as slope. For the dichloroacetate buffers (four dilutions between 5×10^{-3} and 5×10^{-1} M) the intercept value was found to be, within experimental error, zero. No catalysis of hydrolysis was observed with the formate buffers over a 48 h period. In the Table are listed the relative rate constants for H_3O^+ catalysed hydrolysis of (I), methyl-α-D-altropyranoside (II), methyl-α-D-glucopyranoside (III), and methyl-2-deoxy-α-D-glucopyranoside (IV) Examination of the Table shows that (II) hydrolyses at a

Specific acid-catalysed hydrolysis of glycosides

	k_{obs}[a] (s^{-1})	k_{rel}
Methyl-α-D-glycopyranoside[b] (III)	$7{\cdot}60 \times 10^{-7}$	1·0
Methyl-α-D-altropyranoside[c] (II)	$1{\cdot}12 \times 10^{-5}$	16
Methyl-α-D-2,6-anhydroaltropyranoside[d] (I)	$1{\cdot}23 \times 10^{-4}$	162
Methyl-2-deoxy-α-D-glucopyranoside[e] (IV)	$1{\cdot}26 \times 10^{-3}$	1658

[a] k_{obs} values employed were determined in 0·1 M-HCl (pH 1·11) at 70° and μ = 1·0 (with KCl). In all cases the hydrolysis were shown to be specific acid catalysed *via* plots of $\log k_{obs}$ against pH which were linear and of slope of −1·0 between pH 1·0 and 3·0.

[b] Purchased from Pierce Chemical Co. and used without further purification.

[c] Prepared from methyl-α-D-glucopyranoside according to published procedures (see ref. 12).

[d] A sample of this compound sufficient for kinetic runs was kindly provided by Dr. Richtmyer of the Chemical Laboratory, National Cancer Institute, National Institutes of Health.

[e] Kinetic data of ref. 8 extrapolated to 70° *via* Arrhenius plot using rate values at 40°, 49·5°, 60·0°.

rate 16 times that of (III) but that removal of the 2-hydroxyl group increases the rate of hydrolysis of (III) (*i.e.* IV) to a value 100 times greater than that for (II). The rate differential for the H_3O^+ catalysed hydrolysis of (III) and (II) may be attributed to the fact that (II) possesses two axially disposed hydroxy-groups. The relative rates of hydrolysis of methyl-α-D-hexopyranosides parallel the number of axially oriented hydroxyl substituents on the ring. Thus, the relative rates increase 30-fold from methyl α-D-glucopyranoside in its most stable *C*1 conformation to methyl-α-D-idopyranoside, which in the *C*1 conformer would have 3 axial hydroxyl groups and probably does not exist in appreciable amounts in this conformation. The average

relative rate increase is taken to be *ca.* 10-fold per axial hydroxyl group.[6] The increased rate of hydrolysis of 16-fold for (II) compared to (III) is close to the anticipated value of 20. The rate ratio of 1658 for the hydrolysis of (IV) as compared to (III) may be attributed to a change in inductive effect[7] and according to Overend[8] a greater planarity in the C-2, C-1, O, C-5 region for the 2-deoxy-sugar. The specific acid rate constant for (I) should be compared to that of (III) rather than that for (II), because in (I) a strain-free system with minimal interaction of axial hydroxyl groups is obtained. The enhancement of 162 in the rate constant of (I) cannot be attributed to a 2Δ effect nor can it be caused by inductive effects since the hydroxyl and the $-O-CH_2-$ substituents at C-2 have identical σ_I values.[9] The acceleration may then be associated with the restrictive geometry of this glycoside which provides a near planar C-2, C-1, O, C-5 region. However, the magnitude observed is not greater than that which could be expected from other structural variations in a monosaccharide.

Planarity (devoid of intramolecular strain contributions) at the C-2, C-1, O, C-5 region of a methyl glycoside neither changes the mechanism of hydrolysis from $A1$ to S_E2 nor provides an exciting (to the enzymologist) facilitation of hydrolysis. The inability to realize general acid-catalysed hydrolysis of an unactivated glycoside,[10,11] and the inability to realize electrostatic facilitation in the general acid-catalysed hydrolysis of activated glycosides has already been established.[3] The present finding of the relative unimportance (enzymologically speaking) of planarity of the ground state leaves the activation of the substrate by the enforced distortion of the bond angles in the monosaccharide moiety bound to subsite D, as the only remaining untested (in model systems) factor which Vernon considers responsible for the catalytic effect in lysozyme.[13] Rate acceleration, demonstrated in certain acetals, has been attributed to the relief of strain compression;[3] however, the effect of bond distortion in a glycoside is difficult to evaluate due to the lack of pertinent kinetic data. Appropriate models are being investigated to provide this information. The inability to detect general acid-catalysed hydrolysis of (I) provides proof that the rate of glycoside hydrolysis is not dependent on the mole fraction of the protonated half-chair conformation (Equation 1).

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