Stereoelectronic Effects at Oxygen. A Very Large Effect on the Hydrolysis of a Conformationally Locked Acetal: Implications for β-Glycosidase Mechanisms

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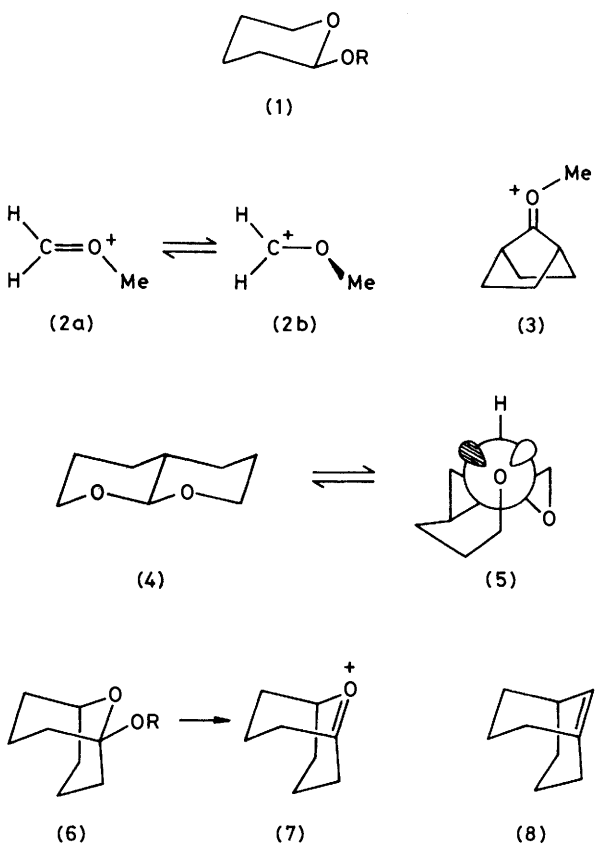
Acetal [6; R = 2,4-$(NO_2)_2C_6H_3$] is rigidly fixed in the equatorial conformation. As a result the lone-pair electrons on the ring oxygen cannot assist C–OR cleavage, and the compound is hydrolysed *ca.* 10^{13} times more slowly than a comparable axial tetrahydropyranyl acetal, allowing an estimate of 19 kcal mol^{-1} for the stereoelectronic barrier to the cleavage of an equatorial tetrahydropyranyl acetal, or β-glucoside, in its ground-state chair conformation. A conformational change, of the sort originally proposed by Phillips and his co-workers for the lysozyme reaction, is thus shown to be an *essential* preliminary to the cleavage of any β-glycoside.

We have shown that the cleavage of an acetal C–O bond proceeds readily only when it is antiperiplanar to one of the non-bonding electron pairs (lone pairs) on the remaining oxygen atom, either in the ground state or in some readily accessible conformation.[1-3] As a result there is a substantial stereoelectronic barrier to the loss of the equatorial OR group from tetrahydropyranyl acetals (1); though this has an observable effect on reactivity only in conformationally fixed systems.[1-3]

In two such systems [2,3] where the conformation is locked by a *trans*-ring junction at the acetal centre, the additional barrier attributable to this stereoelectronic effect can be as large as 7 kcal (30 kJ) mol^{-1}, but the stereoelectronic barrier to acetal cleavage is potentially much larger than this. Its magnitude is given, to a close approximation, by the difference in energy between the planar and perpendicular conformations of the oxocarbenium ion intermediate. This difference has recently been calculated [4] for the methoxymethyl cation (2) as 20.8 kcal (87 kJ) mol^{-1}; and the barrier to rotation in cation (3) has been shown experimentally to be greater than 18.4 kcal (77 kJ) mol^{-1}.[5]

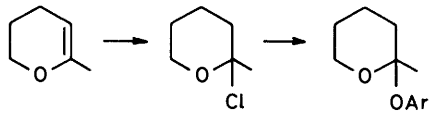
A barrier of this magnitude is so large that observed reactions involving the apparent loss of an equatorial leaving group from an acetal containing the structural unit (1) must be finding their way round, rather than over it. This is presumably what happens in β-glycosidase reactions (see below), and in the two systems we have studied, which are locked by *trans*-ring junctions.[2,3] The flexible ring of the boat-chair form of an acetal (4) of this sort can be twisted into a conformation (5) which, although it suffers from substantial strain [relieved to some extent by the anomeric effect,[6,7] which stabilises conformation (5) [1] selectively] can now react with assistance from the antiperiplanar lone pair [shaded in (5)] and thus avoid the stereoelectronic barrier altogether.

We have discussed this problem in some detail in an earlier paper,[1] and concluded that to explore the magnitude of the stereoelectronic barrier itself, we need to work with conformationally very rigid systems. Probably the most rigid acetals readily accessible are derivatives (6) of 9-oxabicyclo[3.3.1]-nonane; which would be converted by the loss of the OR group into the oxocarbenium ion (7). This species is fixed by the bicyclic system in the perpendicular geometry (2b) across the $C{=}\overset{+}{O}$ bond, corresponding to minimum overlap, and is isoelectronic with the bridgehead olefin (8), which has the highest olefin strain of a large series of known and unknown bridgehead olefins calculated by force-field methods by Maier and Schleyer.[8] So we prepared the 2,4-dinitrophenyl derivative of (6) (in order to minimise the possibility of cleavage of the endocyclic acetal C–O bond [1]), and report here its quite extraordinary lack of reactivity.[9]



Experimental

1-(2,4-Dinitrophenoxy)-9-oxabicyclo[3.3.1]nonane [6; R = 2,4-$(NO_2)_2C_6H_3$] was prepared from the hemiacetal (6; R = H) [10] by arylation with 1-fluoro-2,4-dinitrobenzene. The hemiacetal (1 g, 7 mmol) was dissolved in dry tetrahydrofuran (15 ml) at room temperature, and n-butyl-lithium (5 ml of a 1.6M solution in hexane) added dropwise under nitrogen. The solution was stirred a further 15 min, then 1-fluoro-2,4-dinitrobenzene (3.91 g, 21 mmol) in dry tetrahydrofuran (5 ml) added. The mixture was heated to reflux for 3 h, then



(10) (11) (9)

cooled and poured into water (50 ml). The suspension was extracted with CH_2Cl_2, and the extract washed with 1M-NaOH, then water, dried ($MgSO_4$), and concentrated under reduced pressure to a brown oil, which solidified on standing. Recrystallisation from petroleum (b.p. 40—60 °C)–methanol (2 : 1) gave [6; R = 2,4-$(NO_2)_2C_6H_3$] (1 g, 46%) as pale yellow plates, m.p. 148—149 °C, $\nu_{max.}$ 1 600, 1 540, and 1 380 cm^{-1}, δ($CDCl_3$; 60 MHz) 8.60 (1 H, d, *J* 3 Hz), 8.35 (1 H, dd, *J* 12 and 3 Hz), 8.00 (1 H, d, *J* 12 Hz), 4.55 (1 H, m, O–C–H), and 2.50—1.40 (12 H, envelope), *m/z* 308 (0.1%, M^+), 206 (20), 184 (25), 141 (10), and 125 (100) (Found: C, 54.3; H, 5.35; N, 8.95%; M^+, 308.1015. $C_{14}H_{16}N_2O_6$ requires C, 54.55; H, 5.25; H, 9.1%; *M*, 308.1014). The structure of this compound has also been confirmed by a single-crystal *X*-ray structure determination.[11]

2-Aryloxy-2-methyltetrahydropyrans (9) could not be prepared by the addition of the phenol to the dihydropyran (10), either directly or in the presence of acid catalysts. The successful route involved the reaction of the sodium salt of the phenol with 2-chloro-2-methyltetrahydropyran (11).

Dry HCl was passed through 6-methyl-3,4-dihydro-2*H*-pyran [12] (10) (1.2 g, 12 mmol) for 15 min at 0 °C. Distillation at reduced pressure gave the crude chloride (11) (1.08 g, 80%), b.p. 44—46 °C at 20 mmHg, as a moisture-sensitive liquid which was used without further purification, δ($CDCl_3$) 3.90 (2 H, m) and 2.20—1.50 (6 H, m), on which is superimposed δ 1.80 (3 H, s).

Sodium hydride (2.4 mmol, 115 mg of a 50% dispersion in oil) was washed three times with dry pentane (5 ml) and suspended in dry tetrahydrofuran (3 ml). The slurry was cooled to 0 °C and a solution of the phenol (2.0 mmol in a 2 ml dry tetrahydrofuran) added dropwise. After 10 min, 2-chloro-2-methyltetrahydropyran (11) (2.0 mmol, 270 mg) was added, and the mixture stirred for 2 h as it warmed to room temperature. Filtration through Celite, evaporation of the solvent and p.l.c. on alumina plates (eluant 20 : 80 ether–hexane) gave the desired 2-aryloxy-2-methyltetrahydropyrans, as follows: *2-phenoxy-2-methyltetrahydropyran* (9; Ar = Ph), 220 mg (58%) of an oil, $\nu_{max.}$ (liquid film) 2 950, 1 600, 1 580, and 1 500 cm^{-1}, δ($CDCl_3$) 7.35—7.05 (5 H, m), 4.15—3.70 (2 H, m), 2.10—1.55 (6 H, m), and 1.35 (3 H, s), *m/z* 192 (1.4%, M^+) and 99 (100) (Found: M^+, 192.1141. $C_{12}H_{16}O_2$ requires *M*, 192.1134); *2-(3-bromophenoxy)-2-methyltetrahydropyran* (9; Ar = 3-BrC_6H_4), 370 mg (68%) of an oil, $\nu_{max.}$ (liquid film) 2 950, 1 600, 1 580, and 1 500 cm^{-1}, δ($CDCl_3$) 7.40 (1 H, s), 7.15 (3 H, s), 3.85—3.65 (2 H, m), and 2.05—1.35 (6 H, m) on which is superimposed δ 1.30 (3 H, s), *m/z* 272, 270 (0.52, 0.44%, M^+), and 99 (100) (Found: M^+, 270.0256. $C_{12}H_{15}BrO_2$ requires *M*, 270.0255); *2-(3-nitrophenoxy)-2-methyltetrahydropyran* (9; Ar = 3-$NO_2C_6H_4$) is an unstable liquid, which decomposed on attempted purification by t.l.c. The unpurified mixture from the preparation, after evaporation of the solvent, was used for the kinetic experiments, *m/z* 237 (0.3%) 222 (0.4), and 99 (100) (Found: M^+, 237.0985, $M - 15$, 222.0774. $C_{12}H_{15}NO_4$ requires *M*, 237.1002, $M - 15$, 222.0782).

Results

Reactions were followed by monitoring the release of the substituted phenolate anion, as previously described.[1]

Table 1. Rate constants for the hydrolysis of 1-(2,4-dinitrophenoxy)-9-oxabicyclo[3.3.1]nonane, in 0.1M-TRIS buffer in 50% aqueous dioxane at ionic strength 0.1M (KCl)

pH	*T*/°C	$k_{obs.}/s^{-1}$
8.42	83	7.82×10^{-8}
8.42	83	6.28×10^{-8}
8.42	100	3.74×10^{-7}
8.43	100	4.28×10^{-7}
8.42	117	2.21×10^{-6}
8.42	117	2.57×10^{-6}
8.99	117	2.11×10^{-6}
8.28	117	2.89×10^{-6}
8.06	117	1.96×10^{-6}
7.77	117	1.75×10^{-6}
7.77	117	2.24×10^{-6}

$\Delta H^{\ddagger}$ 27.6 ± 0.2 kcal (115 kJ) mol^{-1}
$\Delta S^{\ddagger}$ −14.1 ± 1.3 cal (60 J) K^{-1} mol^{-1}

Table 2. Rate constants for the hydrolysis of 2-aryloxy-2-methyltetrahydropyrans (9) in 50% aqueous dioxane at 39 °C and ionic strength 0.1M (KCl)

Ar	Conditions (no. of runs)	$k_{obs.}/s^{-1}$
Ph	0.01M-NaOH (2)	$8.05 \pm 0.18 \times 10^{-5}$
Ph	0.01M-NaOH in water (2) [a]	$1.32 \pm 0.04 \times 10^{-2}$
3-BrC_6H_4	0.01M-NaOH (2)	$1.39 \pm 0.01 \times 10^{-3}$
3-$NO_2C_6H_4$	0.01M-NaOH (3)	$9.26 \pm 0.38 \times 10^{-3}$
3-$NO_2C_6H_4$	Carbonate buffer (2) [b]	$8.64 \pm 0.27 \times 10^{-3}$

[a] In water, ionic strength 1.0M (KCl). [b] 0.02M-carbonate–hydrogen carbonate, 50% free base, pH 11.38.

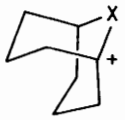
Standard conditions were 50% aqueous dioxane at ionic strength 0.1M (KCl). The rapid hydrolyses of the 2-aryloxy-2-methyltetrahydropyrans were followed at 39 °C in 0.01M-NaOH (at 287, 295, and 392 nm for the phenyl, 3-bromo- and 3-nitro-phenyl acetals, respectively), but the very slow hydrolysis of the bicyclic acetal [6; R = 2,4-$(NO_2)_2C_6H_3$] could only be observed at all at temperatures near 100 °C. Measurements were made by the initial-rate method over a range of temperatures from 83 to 117 °C, using separate sealed tubes for each point. In the case of this very slow reaction aromatic nucleophilic substitution by the attack of OH^- on the aromatic ring is a likely side-reaction. Since this side-reaction would be first order in hydroxide, the problem was minimised by working near pH 8 (TRIS buffer). Under these conditions hydrolysis was pH independent (over the range pH 7.77—8.99), as expected for the spontaneous cleavage of the acetal, but not for its alkaline hydrolysis.

Rate constants and derived data are listed in Tables 1 and 2.

Discussion

The fact that [6; R = 2,4-$(NO_2)_2C_6H_3$] can be prepared at all shows that it is an exceptionally stable acetal. We have never been able to make 2-(2,4-dinitrophenoxy)tetrahydropyran, which is expected to be cleaved very rapidly in its ground state, axial conformation (the half-life predicted from our linear free-energy relationship [13] is *ca.* 1 s in water at 39 °C), and an additional alkyl substituent at the acetal centre, as in (6), would increase reactivity still further. Yet the equatorial 2,4-dinitrophenyl compound (6) is so stable that its hydrolysis can only be followed at elevated temperatures.

To compare the rate of hydrolysis of this very stable acetal



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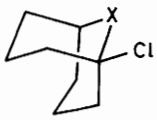

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with that of an appropriate axial compound we have used two extrapolations.

From the Arrhenius plot obtained by measuring the (pH-independent) rate of hydrolysis of [6; R = 2,4-$(NO_2)_2C_6H_3$] over the range 83—117 °C the rate constant for its hydrolysis at 39 °C could be calculated as 2.6×10^{-10} s^{-1}. The simplest appropriate axial compound for comparison is the corresponding 2-aryloxy-2-methyltetrahydropyran (9). These turn out to be remarkably reactive acetals, hydrolysed *ca.* 10^3 times faster than the parent 2-aryloxytetrahydropyrans, so that even the *m*-nitrophenyl compound can be prepared only with difficulty. But rates of hydrolysis could be measured for this, and the *m*-bromo and unsubstituted compounds (9), and shown to fit the Hammett and Brönsted equations (ρ 2.9 ± 0.2, compared with 2.7 ± 0.1 for the series of 2-aryloxytetrahydropyrans measured previously; [13] and β 1.29 ± 0.11, respectively). The latter correlation allows extrapolation to pK_a 4.11 (for the conjugate acid of the 2,4-dinitrophenoxide leaving group).

This gives an estimate of 3.3×10^3 s^{-1} for the rate constant for hydrolysis of [9; Ar = 2,4-$(NO_2)_2C_6H_3$] at 39 °C, 1.3×10^{13} times faster than that estimated for [6; R = 2,4-$(NO_2)_2C_6H_3$].

This enormous difference in reactivity, between an axial tetrahydropyranyl acetal and the comparable compound with the leaving group fixed equatorial, can only reasonably be explained as a stereoelectronic effect. There is little if any strain associated with the formation of the bridgehead cation (12; X = CH_2): [14] the solvolysis of 1-chlorobicyclo[3.3.1]-nonane (13; X = CH_2) in 60% aqueous ethanol [10] is only 60 times slower than that of t-butyl chloride,[15a] and this factor can be accounted for in terms of steric inhibition of solvent assistance [15b] to the ionisation of the bicyclic halide.

Thus we conclude that π-donation by the bridgehead oxygen is indeed suppressed by the geometry about the developing C=$\overset{+}{O}$ bond of (7) so efficiently that the structure of the cation is better represented as (12; X = O).* The dominant effect of the adjacent oxygen is therefore destabilising σ-withdrawal. [Quinn and Wiseman [10] have shown that the solvolysis of (13; X = O) is *ca.* 3 times slower than that of (13; X = CH_2) in 60% aqueous ethanol.]

Meyer and Martin [16] have correlated rates of solvolysis for the series of chlorides (13; X = CH_2, O, S, and NMe) by a modified Hammett–Taft treatment, using for X an effective substituent constant $\sigma_{eff} = \sigma_I + \delta\sigma_{R^+}$. Their best value of the coefficient δ, which is a measure of the fractional efficiency of π-donation by X, was 0.35. In our comparison of related axial and equatorial acetals the inductive (σ-acceptor) effect of the ring oxygen, which is (presumably) independent of geometry, cancels out: so that the difference in reactivity between [6 and 9; Ar = 2,4-$(NO_2)_2C_6H_3$] is almost entirely a measure of the relative efficiencies of π-donation in the two transition states leading to C–OAr cleavage. We can take the maximum possible difference as the 20.8 kcal (87 kJ) mol^{-1} calculated [4] for parallel *versus* perpendicular conformations of the methoxymethyl cation (see above). The observed difference in reactivity of 1.3×10^{13} corresponds to a difference in free energy of activation of 18.4 kcal (77 kJ) mol^{-1}, so that the transition state for the cleavage of the equatorial acetal (6) is 19.1 kcal (80 kJ) mol^{-1} higher in energy than that for the axial compound [9; Ar = 2,4-$(NO_2)_2C_6H_3$] [which is stabilised by the anomeric effect,[7] estimated as at least 0.7 kcal (3 kJ) mol^{-1} in this system].

This amounts to one of the largest stereoelectronic effects on reactivity ever observed, and suggests that π-donation is almost completely (>90%) suppressed in cation (7): and thus that the magnitude of the effect on the cleavage of the C–OR bond of (6) does not represent primarily a conformational barrier, but is a direct measure of the stereoelectronic effect in this system.

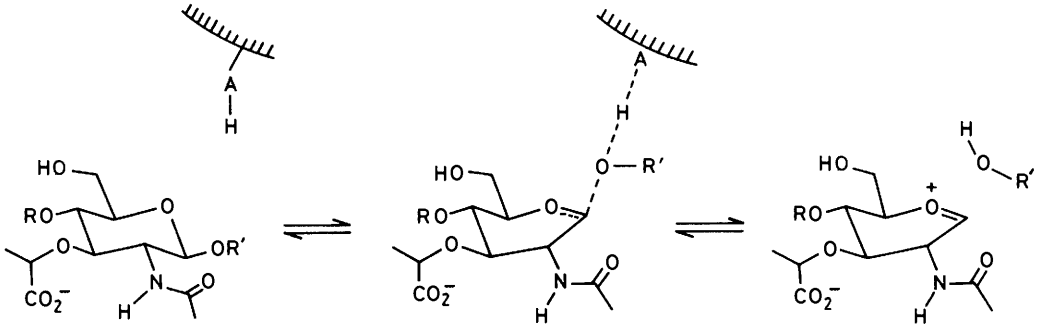
It is important to make a distinction between the very large stereoelectronic effect apparent in this system (6), where the transition state for C–O cleavage is very high in energy primarily because the oxocarbonium ion intermediate is itself a high-energy species, and the smaller effects (up to 7 kcal mol^{-1}) on transition states observed in the conformationally locked systems described in the two preceding papers.[2,3] In those systems conformational adjustment and hence unrestricted π-donation by the ring oxygen is possible once the C–OAr bond is broken, so that both axial and equatorial isomers give rise to stabilised, (almost) identical, oxocarbenium ions. In such cases, as we have discussed above, the difference in transition-state energy for the cleavage of axial and equatorial C–O bonds cannot be very large: it must always be less than the activation energy for the slower of the reverse reactions, which is the addition of a nucleophile to an oxocarbenium ion and thus intrinsically rapid. To measure the full potential of the stereoelectronic effect at oxygen, rather than what is basically a conformational barrier, we have to use a rigid system such as (6). An inevitable consequence is that the axial and equatorial compounds we are able to compare can no longer be simple isomers.

Implications for the Cleavage of β-*Glycosides.*—Our interest in stereoelectronic control of acetal cleavage arose initially from work on the enzyme lysozyme. Lysozyme catalyses the hydrolysis of a polysaccharide constituent of bacterial cell walls, cleaving a β-glycosidic linkage with retention of configuration. Structural and binding studies, summarised in ref. 17, indicate an extended binding site, capable of accommodating oligosaccharides containing up to six sugar residues, as long as one of them, the fourth sugar from the non-reducing end, is twisted into the half-chair (or sofa [18]) conformation. This is significant because the reaction involves the cleavage of the glycosidic bond of precisely this sugar residue; and C–OR cleavage is expected to go through a transition state (15), and probably also an oxocarbenium ion intermediate (16) with conformations close to the half-chair.

The evidence for this picture of catalysis by lysozyme is extensive and self-consistent, and has strongly influenced ideas on the utilisation of binding energy in enzyme catalysis.[19] Recently, however, a number of authors have questioned the importance,[20] and in some cases the existence,[21,22] of substrate distortion on binding, on the basis of calculations which suggest that a hexasaccharide could bind to the active site of lysozyme with all six pyranose rings in the ground-state chair conformation.

Our results show quite clearly that a β-glycoside held in the ground-state chair conformation will not react at all. As with our conformationally fixed acetal (6), the lone pairs on the ring oxygen of a β-glycoside [*e.g.* (14)] cannot overlap significantly with σ^*_{C-OR} because antiperiplanar to the C–OR bond are two ring bonds. The stereoelectronic barrier to C–OR cleavage of a system such as (14) held in the equatorial conformation [*ca.* 19 kcal (80 kJ) mol^{-1}] is so large that it is inconceivable that any readily observed reaction, particularly

* This effect has been described in the past as steric inhibition of resonance. See Meyer and Martin [16] for leading references.



the rapid C–OR cleavage catalysed by an enzyme, should go over it. The alternative is an energetically relatively inexpensive conformational change about the (ring) O–C(OR) bond. Lone pair–σ^*_{C-OR} overlap becomes gradually more efficient as the ring flattens and approaches the half-chair or sofa conformation, whereas our crystallographic results [23] suggest that even the very early stages of C–OR cleavage are suppressed if no overlap is possible. Thus conformational change is an essential preliminary to the cleavage of a β-glycoside (or other equatorial tetrahydropyranyl acetal [1]), and every enzyme-catalysing β-glycosyl transfer must make provision for it. {This is true whether the mechanism involves an oxocarbonium ion [*e.g.* (16)] as a full intermediate, or participation by a nucleophile in C–OR cleavage. S_N2 reactions at acetal centres are much faster than displacements of comparable leaving groups from related ethers because the transition state for the concerted process can be stabilised by lone-pair donation from the 'spectator' oxygen,[24] so that the stereoelectronic requirements for the concerted and stepwise mechanisms are expected to be similar.}

In the lysozyme reaction this essential conformational change could be part of the initial binding process, as originally suggested, or it could occur during one or more of several subsequent isomerisation steps which can be detected by fast-reaction techniques.[25] It is also in principle possible that it is one of a group of processes, culminating in C–OR cleavage, which make up the rate-determining step, and cannot be separated experimentally.[25,26] As in the hydrolysis of a β-glycoside (or other conformationally flexible equatorial tetrahydropyranyl acetal) free in solution, the energy required to raise the substrate to a higher energy reactive conformation can be recouped, as long as the conformational barrier concerned is significantly smaller than the energy of activation for the cleavage reaction.[1] The $\Delta G^{\ddagger}$ value for the lysozyme-catalysed cleavage of chitohexose [almost 20 kcal (84 kJ) mol^{-1}] [25] is unusually high for an enzyme reaction, and is in fact substantially greater than the conformational barrier involved.

We have shown in this investigation [1-3] that a conformational change of the sort proposed originally by Phillips and his co-workers [17] is an essential preliminary to the cleavage of any β-glycoside.[27] Only further work on individual enzymes can show at what stage this conformational change occurs in each case.

Acknowledgements

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