On the antiperiplanar lone pair hypothesis and its application to catalysis by glycosidases

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The idea that sp^3 lone pairs of electrons on a heteroatom direct the departure of a leaving group from an adjacent tetrahedral carbon centre is shown to be untenable as a general principle, and the permissive evidence in favour of it to be accounted for by the principle of least nuclear motion. The application of the idea to the majority of glycopyranosidases, which work by a double-displacement mechanism, is shown to require implausible contortions of the pyranose ring.

Deslongchamps (1975, 1977) proposed that the departure of a leaving group from a tetrahedral carbon atom, substituted with one or more heteroatoms, is assisted in an additive way by antiperiplanar lone pairs of electrons on the heteroatoms. This was termed the 'theory of stereoelectronic control' and the idea was conflated with the well-recognized preferences of intermediates for equilibrium geometries different from those of starting materials (Deslongchamps, 1983). The present paper addresses itself only to the original hypothesis which is termed, for reasons of semantic precision, the 'antiperiplanar lone pair hypothesis' (ALPH). The purpose of this communication is to summarize the serious conceptual and experimental problems with ALPH in general, and to show that in its application to enzymic and non-enzymic glycosyl transfer it is untenable.

Perrin & Arrhenius (1982) pointed out that all of Deslongchamps' data in support of ALPH to that date was permissive, rather than compelling, since it was based entirely on experiments in which either the thermodynamically more stable reactant of a pair of stereoisomers failed to react, or the thermodynamically more stable of a pair of possible products was produced from a common intermediate. In logic, therefore, no new phenomenon needed to be adumbrated.

A further conceptual flaw in ALPH is that it is a statement about reactive ground state conformations. Thus, Deslongchamps (1983, p. 39) states that " α -glycosides must hydrolyze via their ground

state conformation whereas β -glycosides must first assume a boat conformation in order to fulfill the stereoelectronic requirement" (original italics). The idea is illustrated in Fig. 1 for glucosides.

Now, it is an axiom of transition state theory that information can be obtained only about ground states and transition states: in cases like the present where rapid conformational pre-equilibria are supposedly involved, this axiom becomes the Curtin–Hammett principle (reviewed by Seeman, 1983). Therefore, Deslongchamps' statement, insofar as it pertains to reactions in free solution, is wholly untestable. Only in the context of enzyme catalysis is it scientific (i.e. in principle disprovable), since kinetically accessible ES complexes, present in appreciable concentration, may contain the substrate in a conformation different from that in free solution.

There are a number of experimental difficulties with ALPH; results with cyclic orthoesters were originally rationalized on the assumption that the lifetime of a hemiorthoester was short compared with the time of rotation about C–O bonds. With the direct detection of such intermediates (Capon *et al.*, 1981; McClelland & Santry, 1983) this assumption has become untenable; moreover, some of the original experiments were shown to be wrong (Capon & Grieve, 1982). Nonetheless, it has proved possible to rationalize the correct experimental data (on mild acid hydrolysis, conformationally labile cyclic orthoesters such as **I** give a mixture of lactone and hydroxyester but conformationally rigid ones such as **II** give hydroxyester exclusively) by invoking a 'secondary stereoelectronic effect' as well as a primary one (Deslongchamps, 1983, pp. 72–78).

Abbreviation used: ALPH, antiperiplanar lone pair hypothesis.

Fig. 1. Pathways for hydrolysis of glucopyranosides as predicted by ALPH

Fig. 2. Illustrating atomic trajectories consequent upon hydride abstraction by O_3 from the anomeric centre of glycosides. To attain the planar conformation of the dioxocarbonium ion, the $-OCH_3$ group must rotate through 90° about the C-1-O-5 bond in the case of an α -glycoside, but only through 30° in the case of a β -glycoside.

The one compelling demonstration of the existence of a phenomenon of some sort related to ALPH is the detection of ω -aminoamides as kinetic products, and lactams as thermodynamic products, in the hydrolysis of 5- and 6-membered cyclic amidines (Perrin & Arrhenius, 1982). We have since shown (Hosie *et al.*, 1984) that this result is also predicted by the principle of least nuclear motion. Arguments exactly analogous to those presented in Hosie *et al.* (1984) immediately

explain the results with cyclic orthoesters, without any further *ad hoc* hypotheses. Least motion arguments also readily explain much of the permissive evidence in favour of ALPH, such as the lability of β -, but not α -, glycosides to ozone (Fig. 2) (*cf.* Deslongchamps, 1983, pp. 41-47).

Least motion effects (reviewed by Hine, 1977) are small, elusive, and apply most powerfully to reactions having central transition states. They are easily over-ridden by other steric effects: therefore

it is not surprising that nucleophilic attack on penicillins occurs from the α -face, contrary to ALPH; attack in an ALPH sense would yield a strained *trans*-fused bicyclo [3:2:0] system (Page, 1984).

The non-enzymic hydrolyses of glycosides and acetals pass through late transition states; therefore, if phenomena attributed to ALPH are indeed merely least motion effects, then ALPH will apparently not apply to acetal and glycoside hydrolyses. In fact, Chandrasekhar *et al.* (1983) found that the equatorial epimers of pairs of conformationally restricted acetals were about 3-fold more reactive than their axial epimers, in apparent flat contradiction to ALPH. This pattern of reactivity is entirely accounted for by the operation of the anomeric effect on ground state stability. Nonetheless, Chandrasekhar *et al.* (1983) chose to rationalize their data in terms of ALPH and reaction through non-chair conformers. As explained above, this rationalization violates the Curtin-Hammett principle, but it is possible, if assumptions are made about the relationship between transition state conformation and reactive ground state conformation, to examine the supplementary hypothesis of reaction through non-ground-state conformers using the leaving group as a conformational determinant. We have done this with anomeric pairs of glycosyl pyridinium salts, and have shown the supplementary hypothesis to be untenable (Hosie *et al.*, 1984).

Despite these results with simple acetals, Briggs *et al.* (1983), on the basis of the inertness of compound **III** to spontaneous hydrolysis, stated that (a substrate conformation-change) is an "essential preliminary to catalysis by β -glycosidases" (original italics). This statement is a *non sequitur* arising from confusion of ALPH with the long-recognized preference of oxocarbenium ions for a planar conformation: the perpendicular configuration of $\text{CH}_2=\text{O}^+-\text{CH}_3$, with the methyl group out of the plane defined by the CH_2 group

and the oxygen atom, lies at least 11.9 kcal/mol (49.7 kJ/mol) (Fărcașiu *et al.*, 1979) above the favoured planar conformation. Compound **III** necessarily gives rise to a perpendicular oxocarbenium ion. Departure of aglycone from a β -glycopyranoside in the $^4\text{C}_1$ conformation will give a non-planar oxocarbenium ion only if the motion of the anomeric hydrogen is restricted so that it cannot become coplanar with C-2, C-1, O-5 and C-5 in the glycosyl cation. There has never been any suggestion of this.

Studies of real glycosidases provide no evidence for ALPH; indeed, rather the contrary. Moreover, most glycosidases work by a double displacement mechanism and give sugars of the same anomeric configuration as the glycoside substrate. Application of ALPH to both steps of the catalytic sequence requires implausible contortions of the pyranose ring, especially in the case of 'retaining' β -glycosidases.

Warshel & Levitt (1976) accounted theoretically for the catalytic power of lysozyme without taking any sort of prior substrate distortion into account. The X-ray crystallographic studies of Ford *et al.* (1974) on the binding of the aldono- δ -lactone derived from tetra-*N*-acetylchitotetraose to lysozyme were interpreted in terms of binding of the D ring in a sofa conformation, in which there was distortion towards the half-chair conformation of the putative oxocarbenium ion intermediate. (These authors' data were also compatible with the $\text{B}_{3,0}$ conformation of the D ring, but this was rejected on the grounds the lactone function was non-planar.) Distortion towards planarity of the oxocarbenium ion intermediate or oxocarbenium-ion like transition state there must be, but whether this occurs during binding or catalysis is not germane to considerations of ALPH.

Studies on a number of β -glycosidases have so far failed to uncover a discrete ring-distorting kinetic event. However, recently evidence has been presented for such an event in catalysis by yeast α -glucosidase (Hosie & Sinnott, 1985) and we have suggested that the reactive conformation is the $^{2,5}\text{B}$ classical boat conformation: i.e. presented with a substrate which in the preferred $^4\text{C}_1$ conformation has a lone pair of electrons antiperiplanar to the leaving group, the enzyme distorts it to the $^{2,5}\text{B}$ conformation, where no such lone pair is available, before performing bond cleavage. The $^4\text{H}_3$ and $^{2,5}\text{B}$ conformations of D-glucono- δ -lactone lie close to one another in energy (Walaśzek *et al.*, 1982) and it is reasonable to assume the conformational preferences of the glycosyl cation are similar to those of the lactone. It is possible that other α -glucosidases work via transition states in which the pyranose ring is close to the $^4\text{H}_3$ conformation. Thus, the indolizidine alkaloid castanospermine,

III

which can mimic the glucopyranosyl cation in the 4H_3 but not in the ${}^{2,5}B$ conformation (Hosie & Sinnott, 1985), does not inhibit yeast α -glucosidase but is a powerful inhibitor of the mammalian lysosomal enzyme (Saul *et al.*, 1983). There are thus some indications that α -glucosidases can evolve indifferently in an ALPH and anti-ALPH sense.

Where 'retaining' glycosidases have been examined they have been found to possess an active-site carboxylate group (see Fowler & Smith, 1983; Herrchen & Legler, 1984, for recent examples). In the ES complex this carboxylate group is on the opposite side of the sugar ring to the aglycone (Hehre *et al.*, 1977; Lalgerie *et al.*, 1982). The linkage between the glycosyl moiety and the active site carboxylate must be largely covalent, for two reasons. Firstly, rate constants for hydrolysis of glycosyl-enzyme intermediates are in the range 10 – 10^3 s^{-1} (Viratelle *et al.*, 1969; De Bruyne *et al.*, 1979; Weber & Fink, 1980; Li *et al.*, 1983), whereas it is unlikely that the glucosyl cation in water has a lifetime longer than, say, 10^{-9} s (Young & Jencks, 1977; Sinnott & Jencks, 1980). Secondly, α -deuterium kinetic isotope effects (k_H/k_D) on deglycosylation are greater than unity, whereas if the glycosyl-enzyme were ionic effects less than unity would be observed. [Effects of 1.25 for *lacZ* β -galactosidase (Sinnott & Souchart, 1973) 1.10 for *ebg*^a, *ebg*^b and *ebg*^{ab} β -galactosidase (D. Holdup & M. L. Sinnott, unpublished work), all from

Escherichia coli, and 1.11 for β -glucosidase of *Stachybotrys atra* (van Doorslaer *et al.*, 1984) are known.]

Microscopic reversibility, combined with ALPH, would require attack of a nucleophile on an oxocarbenium ion to generate an antiperiplanar lone pair of electrons (Beaulieu *et al.*, 1980). Therefore, if the dictates of ALPH were to be followed in the course of the first chemical step of a retaining β -glycosidase, occurring via either a discrete oxocarbenium ion or an oxocarbenium-ion-like transition state, then the following changes in ring conformation must occur (Fig. 3).

(i) Ground state 4C_1 to a conformation with an antiperiplanar lone pair of electrons. Work with anomeric pairs of glucopyranosyl pyridium salts has indicated that the 1S_3 conformation is probably the lowest energy conformation with a lone pair of electrons antiperiplanar to a β leaving group (Hosie & Sinnott, 1985).

(ii) 1S_3 reactive ground state to (say) $B_{2,5}$ conformation of the oxocarbenium ion. Conversion of the 1S_3 to the $B_{2,5}$ or ${}^{2,5}B$ conformation involves a change of 90° in pseudorotational angle.

(iii) Change in the conformation of the ion so that α attack by the active-site carboxylate will generate an antiperiplanar lone pair in the glycosyl enzyme, say to 4H_3 .

(iv) Attack by the active-site carboxylate on the α face to give a 4C_1 glycosyl enzyme.

Fig. 3. Conformational changes required by ALPH of the first chemical step of a 'retaining' β -glycosidase

It could be argued that loss of aglycone from the 1S_3 conformation would generate the 4H_3 conformation directly by a least motion path, but this probably would be energetically less favourable. Moreover, the least motion path from the 4C_1 to the 1S_3 conformation would go through the 4H_3 conformation of the oxocarbenium ion, and thus an oxocarbenium-ion-like transition state could be attained directly without the system going through a conformation in which an antiperiplanar lone pair would be presented to the leaving group.

Deglycosylation will be the reverse of the formation of the glycosyl enzyme, and so for ALPH to apply to a retaining β -glycosidase four substantial changes of ring conformation ($^4C_1 \rightarrow ^1S_3$; $B_{2,5} \rightarrow ^4H_3$; $^4H_3 \rightarrow B_{2,5}$; $^1S_3 \rightarrow ^4C_1$) would have to be accommodated. Similar considerations apply to retaining α -glycosidases, although a β -glycosyl enzyme might in principle keep the pyranose ring in the (say) 1S_3 conformation.

Were ALPH to apply to glycosidase catalysis, therefore, extraordinary conformational subtlety would be required of the active sites of 'retaining' glycosidases. The structural demands of 'inverting', one-step glycosidases would be much less. It is thus very difficult to reconcile ALPH with the observed evolutionary preference for 'retaining', double-displacement mechanisms of glycoside hydrolysis.

ALPH, therefore, cannot profitably be applied to either enzymic or non-enzymic glycoside hydrolysis. It should be abandoned in this area and probably in general.

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