Theoretical modelling of kinetic isotope effects for glycoside hydrolysis in aqueous solution by a hybrid quantum-mechanical/molecular-mechanical method

John A. Barnes and Ian H. Williams*

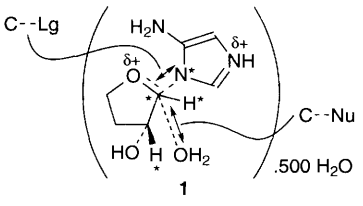
School of Chemistry, University of Bath, Bath, UK BA2 7AY

A hybrid QM/MM computational study of acid-catalysed hydrolysis of a model for adenosine monophosphate solvated by *ca.* 500 water molecules suggests the possibility of both stepwise $D_N{}^*A_N$ and concerted A_ND_N mechanisms, but agreement between calculated and experimental kinetic isotope effects for multiple substitutions indicate the former mechanism.

Glycoside hydrolyses teeter on the brink between stepwise and concerted mechanisms. As the stability of the oxocarbenium ion decreases, so its lifetime diminishes until the concerted mechanism is enforced by the non-existence of the intermediate in the stepwise mechanism. A typical glycosyl cation has an extremely short lifetime (*ca.* 10^{-12} s) in water[1] and does not exist in the presence of anionic nucleophiles.[2] We now report the most significant results of a theoretical simulation of hydrolysis of an *N*-glycoside in aqueous solution, a reaction which appears to occur close to this mechanistic borderline.

Quantum-mechanical (QM) approaches are essential for theoretical modelling of transition-state (TS) structure since molecular mechanics (MM) is unsuitable for predicting processes of electronic reorganisation. Realistic modelling of explicit solvation of chemical reactions involves too many atoms to be treated by conventional QM approaches; however, hybrid QM/MM methods are now becoming available, which combine the merits of a QM treatment of bond making and breaking with a MM description of the environment within which the chemical events occur.

The enormous potential of this QM/MM approach[3] is demonstrated by a study of a model **1** for acid-catalysed hydrolysis of adenosine monophosphate (Scheme 1) embedded within a 15 Å radius sphere of *ca.* 500 water molecules; the QM atoms of the reacting system are treated by the AM1 semiempirical molecular orbital method[4] and the solvent water molecules by the TIP3P empirical potential.[5] The CHARMM22 program[6] was used to obtain the QM/MM energy surface shown in Fig. 1: at each point on a grid, formed by pairs of values for the making bond (C···Nu) to the nucleophilic water molecule and the breaking bond (C···Lg) to the aglycone leaving group at 0.2 Å intervals, relaxation of all remaining coordinates to a local energy minimum was performed. TS structures were located accurately by fine-grained (0.02 Å) grid searches around the approximate saddle points, and were characterized by determination of the vibrational frequencies for the QM atoms within the MM solvent using our own modification of the CHARMM22 code. A solvent boundary potential was employed to prevent evaporation of water molecules from the surface of the sphere, and all non-bonded interactions were included. The simplified model **1** was adopted by analogy with an earlier BEBOVIB study of kinetic isotope effects (KIEs) for this reaction.[7]



1

The topography of the resulting surface reveals a sharp saddle point leading from the reactant valley (Fig. 1, bottom) into a shallow bowl, corresponding to an intermediate (middle left), from which the product valley (top right) is reached by traversing a second broad saddle region; this suggests a stepwise $D_N{}^*A_N$ preassociation mechanism. However, the surface also shows the presence of a separate pathway leading directly to the products by means of a distinct saddle point (middle right) across the ridge from the reactant valley, and thereby suggesting a concerted A_ND_N mechanism. The TSs for

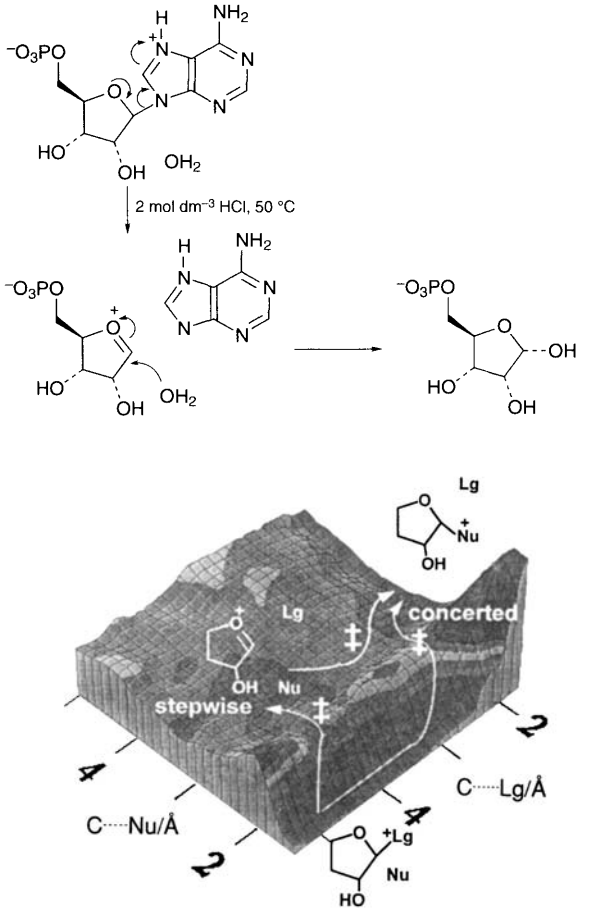


Fig. 1 QM/MM energy surface for model **1** of acid-catalysed hydrolysis of adenosine monophosphate (Nu = water nucleophile; Lg = aglycone leaving group)

the stepwise and concerted mechanisms possess distinctly different structures (Fig. 2) but their energies are very similar ($\Delta E‡ \approx 146$ kJ mol^{-1}, $\Delta\Delta E‡ \approx 0.2$ kJ mol^{-1}); although the looser $D_N{*}A_N$ TS would be slightly favoured entropically, this nonetheless suggests the possibility that both mechanisms could be followed concurrently.

The most subtle but powerful experimental probes for TS structure are KIE measurements. KIEs may also be computed by consideration of the shifts in vibrational frequencies resulting from isotopic substitutions at various atoms of the reacting system,[8] and comparison with experimental values provides a stern test of any TS structure predicted by theoretical modelling. Semiclassical KIEs calculated for each of the TSs on our QM/MM energy surface are given in Table 1 for isotopic substitution at four separate positions. Three of the results agree for both mechanisms almost to within the reported experimental error of the values measured[9] for acid-catalysed hydrolysis of adenosine monophosphate in 2 mol dm^{-3} HCl at 50 °C. These are the primary ^{14}C and ^{15}N effects involving the breaking bond to the leaving group, and the secondary β-^{2}H effect at the 2′ position adjacent to the developing carbocationic centre. The fourth KIE, the secondary α-^{3}H effect at the reaction centre itself (the 1′ position), shows significantly better agreement (again almost to within the reported experimental error) for the TS computed for the stepwise preassociation mechanism than for the concerted mechanism. This finding concurs with the mechanistic interpretation of Schramm and co-workers.[7]

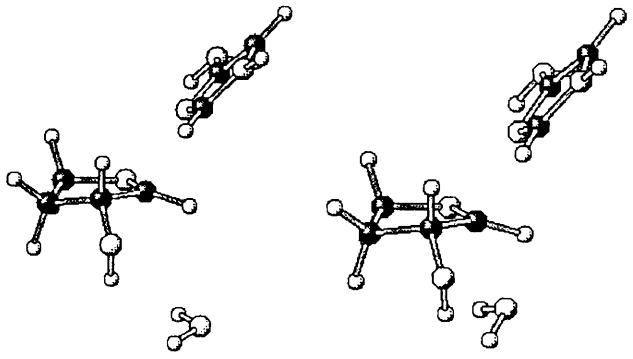


Fig. 2 Computed QM/MM model TS structures for acid-catalysed hydrolysis of adenosine monophosphate: (*a*) stepwise $D_N{*}A_N$ preassociation mechanism; (*b*) concerted A_ND_N mechanism

Table 1 Experimental kinetic isotope effects for acid-catalysed hydrolysis of adenosine monophosphate in 2 mol dm^{-3} HCl at 50 °C and QM/MM calculated semiclassical KIEs for model TS structures for stepwise $D_N{*}A_N$ preassociation and concerted A_ND_N mechanisms

		QM/MM calculated	
Isotope effect	Experimental	Stepwise	Concerted
1° ^{14}C	1.044 ± 0.003	1.045	1.044
1° ^{15}N	1.030 ± 0.002	1.028	1.028
2° β-^{2}H	1.077 ± 0.002	1.074	1.071
2° α-^{3}H	1.216 ± 0.004	1.211	1.164

To our knowledge this is the first report of the use of a hybrid QM/MM method for theoretical calculation of KIEs. In general, theoretical modelling of chemical mechanisms may bear no relation to experiment, but the use of KIEs provides a very strong anchor. A computed TS structure which predicts KIEs for multiple isotopic substitutions in agreement with reliable experimental results is likely to be of real value for rationalization and prediction. The very encouraging agreement found in this preliminary study gives hope that computational modelling with hybrid QM/MM approaches offers a valuable tool, complementary with experiment, capable of providing insight into complex reaction mechanisms.

We are grateful to the EPSRC and to Celltech Research for financial support.

References

1 T. L. Amyes and W. P. Jencks, *J. Am. Chem. Soc.*, 1989, **111**, 7888.
2 N. S. Banait and W. P. Jencks, *J. Am. Chem. Soc.*, 1991, **113**, 7051.
3 M. J. Field, P. A. Bash and M. Karplus, *J. Comp. Chem.*, 1990, **11**, 700.
4 M. J. S. Dewar, E. G. Zoebisch, E. F. Healy and J. J. P. Stewart, *J. Am. Chem. Soc.*, 1985, **107**, 3902.
5 W. L. Jorgensen, J. Chandrasekhar, J. Madura, R. W. Impey and M. L. Klein, *J. Chem. Phys.*, 1983, **79**, 926.
6 B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan and M. Karplus, *J. Comput. Chem.*, 1983, **4**, 187.
7 F. Mentch, D. W. Parkin and V. L. Schramm, *Biochemistry*, 1987, **26**, 921.
8 L. Melander and W. H. Saunders, *Reaction Rates of Isotopic Molecules*, Wiley Interscience, New York, 1980.
9 D. W. Parkin and V. L. Schramm, *Biochemistry*, 1987, **26**, 913.

Received, 30th August 1995; Com. 5/05721C