Stereoelectronic Influence of Fluorine in Enzyme Resolutions of α-Fluoroesters

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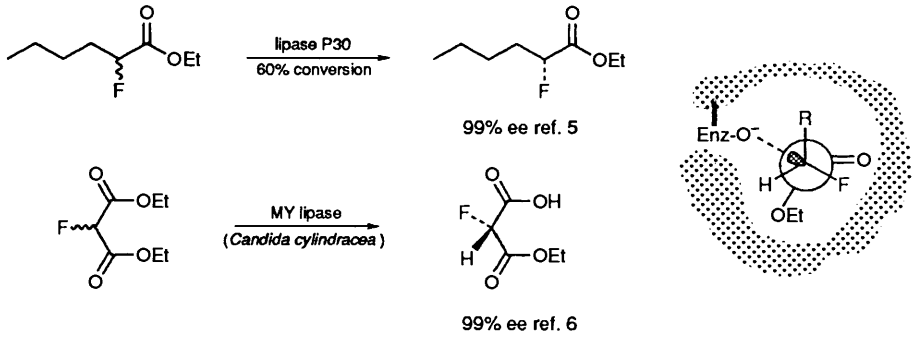
The very high enantioselectivity demonstrated by two different lipase enzymes towards α-fluoroesters is shown from quantitative PM3 and *ab initio* 6-31G* SCF-MO calculations to arise from stereoelectronic factors, which are predicted to be reduced when the diastereoisomeric transition states are modelled in the condensed aqueous phase.

Selectively fluorinated compounds are increasingly being deployed for pharmaceuticals and fine chemicals applications, and the development of new methods towards the generation of homochiral fluorinated compounds is a current challenge.[1] Synthetic access to such compounds, particularly with –F or $-CF_3$ at the stereogenic centre, is limited[2] and in this regard enzymic resolutions[3] and microbial reductions[4] have offered a versatile and successful approach to such systems. In the arena of biotransformations two hydrolytic resolutions of α-fluoroesters have been reported,[5,6] where different lipase enzymes have displayed an almost complete distinction (>99% ee) between hydrogen and fluorine atoms (Scheme 1). When a lipase P30 mediated hydrolysis of racemic ethyl 2-fluorohexanoate was allowed to proceed to 60% conversion the isolated residual ester had predominantly the (*R*)-configuration (99% ee).[5] In the second example,[6] the Amano MY lipase displays a high selectivity towards hydrolysis of the *pro-S* ester group of diethyl fluoromalonate. Although the steric influence of a single fluorine atom has been detected[7] in certain enzyme systems, the perturbation is small and it is difficult to rationalise the selectivity of the lipase resolutions on the basis of steric effects alone. It is our view that the stereoelectronic influence of fluorine in these two bio-transformations should not be underestimated, and may be a controlling factor in the selectivity. For a particular lipase, the serine alkoxide nucleophile will attack at a predesignated face of the ester carbonyl group. The two enantiomers of the α-fluoroester substrate will then give rise to different diastereomeric transition states. We have previously suggested[7] that the favoured diastereoisomer will have the alkoxide nucleophile approaching antiperiplanar to the fluorine atom as illustrated in the staggered transition structure (Scheme 1), such that the alkoxide nucleophile is stabilised by lone pair donation into the σ* orbital (dark lobe) associated with the C–F bond. Clearly if the fluorine and hydrogen atoms are exchanged (for the other substrate enantiomer), then this Ahn–Eisenstein type interaction[8] does not exist. Implicit in this analysis is a requirement for the enzyme to lock the R group such that the transition state conformation can accommodate such a stereoelectronic interaction. We report here quantitative gas phase and solution theoretical models for this reaction which demonstrate that the enantioselectivities observed are entirely consistent with the energy difference between such diastereomeric transition states.

The model reaction between methyl 2-fluoropropionate and methoxide or hydroxide anion was studied at the PM3 or *ab initio* 6-13G* SCF-MO levels respectively, methods which have been successfully applied to stereoelectronic rationalisation of several other reactions.[9]

The calculated discrimination in favour of diastereoisomer **1** (Fig. 1) † is 2.4 or 2.8 kcal mol^{-1} at the PM3 or 6-13G* levels respectively (Table 1). The calculated PM3 entropy difference between **1** and **2** of 0.4 cal K^{-1} mol^{-1} results in a free energy difference of 2.5 kcal mol^{-1} at 300 K. Energy differences of this magnitude correspond to a **1**:**2** ratio of ≈100:1. The enthalpy and free energy differences reduce to 1.3 and 0.7 kcal mol^{-1} respectively when the PM3–COSMO aqueous solvation model[10] is applied, implying a significant reduction in specificity. The origins of these effects are evident in the PM3

† Theoretical calculations were carried out at the restricted Hartree–Fock level (RHF) PM3 semi-empirical method, as implemented in the MOPAC-93 program,[9] using a relative permittivity of 78.4 for water and 60 surface segments per atom for the COSMO model. *Ab initio* calculations were performed using the GAUSSIAN-92 program. All structures were optimised using the eigenvector following algorithm, followed by a vibrational analysis to characterise the transition state. Use of the COSMO option approximately doubles the computation time compared with a gas phase calculation. Computer readable files for Apple Macintosh and Microsoft Windows systems in Quicktime™ video animation format illustrating the three dimensional properties of **1** and **2** are available for general access from the Gopher + server *argon-fddi.ch.ic.ac.uk*. These files will reside in the Royal_Society_of_Chemistry/Perkin_Trans_2/3_05980D directory for a period of at least two years from the publication of this paper. A description of how to visualise such material, together with appropriate programs is available from the same source.



Scheme 1

Table 1 Calculated properties of transition states **1** and **2**

Property	1	2	1 (COSMO)	2 (COSMO)
$\Delta H^{\ddagger}_{PM3}$ [a]	16.4 (−188.3)	18.8 (−185.9)	22.4 (−257.2)	23.7 (−255.9)
$E_{6\text{-}31G^*}$ [b]	−480.0904	−480.0858	—	—
$r_{O\text{-}C}$ [c]	2.14	2.18	2.23	2.23
v_i [d]	229	246	284	282
HOMO [e]	−3.89, −3.75	−3.72, −3.54	−8.72, −8.38	−8.67, −8.32

[a] PM3 Calculated activation enthalpy (enthalpy of transition state), in kcal mol^{-1}. [b] *Ab initio* 6-31G* energy in Hartrees for hydroxide attack (1 Hartree = 627.5 kcal). [c] PM3 Me–O ··· C distance, in Å. [d] PM3 Reaction coordinate mode, in cm^{-1}. [e] PM3 two highest occupied molecular orbital energies, in eV.

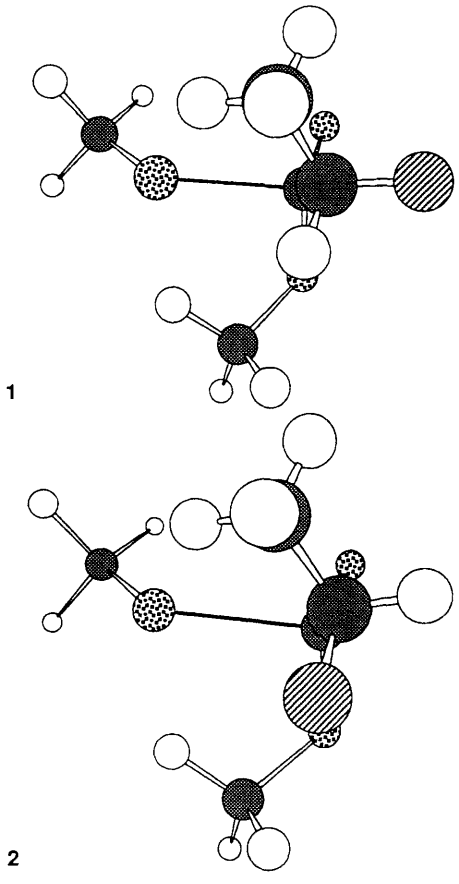


Fig. 1 Calculated PM3 geometry of transition states **1** and **2**

energies of the oxy-anion lone pair HOMO orbitals. Those for **1** ≈0.17–0.21 eV are more stable than **2** as a result of stabilisation by interaction with the σ* orbital, which also induces a small contraction in the O–C forming bond. The orbital energy *differences* are reduced to ≈0.05 eV when the PM3–COSMO model is applied, largely because the oxy-anion orbital is considerably stabilised by the solvation model (Table 1). This in turn increases the energy difference between this orbital and the unoccupied C–F σ* orbital from ≈10 to ≈11.5 eV, thus reducing the perturbational stabilisation. A key feature of lipase chemistry is the intricate proton relay system which has evolved to maximise the nucleophilicity of the alkoxide anion.[11] Attenuation of this nucleophilicity by coordination at the active site is unlikely, and on this basis we argue that the desolvated model more closely reflects the true situation in this case.

Models of the common hydrolytic enzymes (*e.g. Candida cylindracea* lipase,[12] pig liver esterase[13]) have been proposed on the basis of substrate/activity profiles which attempt to map the *steric* constraints of the active sites. In the light of the current study, the efficacy by which individual lipases resolve α-fluorinated esters should afford additional spatial information deduced from the stereoelectronic interactions allowed at that active site.

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