Mechanism and transition state structure for papain catalysed amide hydrolysis, using a hybrid QM/MM potential

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The transition state for amide hydrolysis catalysed by the enzyme papain is determined using a hybrid QM(AM1)/MM potential, showing that the reaction is concerted rather than stepwise, with no involvement of a tetrahedral intermediate.

The concept of a transition state analogue plays a central role in the design of inhibitors of enzyme catalysed reactions and is thus valuable in drug discovery.[1–3] Direct information on the structure of the transition state for reactions involving the natural substrate is difficult to obtain, although in favourable situations crystallographic studies have proved valuable.[4] For the more simple situation of gas phase reactions of small molecules, electronic structure methods can routinely predict stationary structures on the potential energy surface including transition states.[5] Combined quantum mechanical/molecular mechanical (QM/MM) potentials have been used by several groups to model reactions catalysed by enzymes.[6–10] However, most calculations involve single point evaluation, or the prediction of minimum energy structures.

We have implemented such a hybrid potential method coupling the QM code GAUSSIAN94[11] and the MM code AMBER[12] to permit both minimum energy structures *and* transition states to be readily obtained for complex enzyme catalysed reactions. Full details of this implementation will be described in a subsequent publication. In brief, the computational strategy that we have adopted starts with a crystallographic structure of an enzyme–substrate complex, as closely related as possible to the system under investigation, followed by the building and energy minimization, using AMBER, of the system to be modelled. The resulting structure is partitioned into QM and MM atoms, and the corresponding coordinate and topology files used as data to GAUSSIAN94, suitably modified, to carry out the electronic structure calculation employing the hybrid QM/MM potential. We find in general that the QM fragment must extend up to the C_α or C_β atom of each residue of the active site. The valence of the QM fragment is then satisfied by the addition of H atoms to the QM system, and redistribution of the small C_α or C_β charge to surrounding MM atoms. We here report calculations of the mechanism of catalysis by the enzyme papain, employing this protocol, including the first transition state for an enzyme catalysed reaction to be properly characterized.

The mechanism of amide hydrolysis by the enzyme papain, which is considered to be the archetype of cysteine proteases, has been studied both experimentally and theoretically (see ref. 13 for a review). In the resting state of the enzyme, the important residues of the active site (cysteine, histidine) form an ion pair and are subsequently involved in nucleophilic attack and protonation of the amide substrate. We address two key questions concerning this reaction which are relevant for any reaction in which S^- is a nucleophile; does the reaction involve a tetrahedral intermediate or transition state and if so how is it stabilized?

An enzyme–substrate structure was constructed following the approach of Arad *et al.*[14] using an experimentally determined inhibitor–enzyme structure[15] together with a structure of the oxidized enzyme.[16] The solvated structure (99 water molecules) involving the substrate Ace-Phe-Ser-Ile-Nme (Ace is an acetyl group and Nme is an *N*-methyl group) was energy minimized (using AMBER 4.0 force field). This substrate was then replaced by *N*-methylacetamide and the system modelled using a hybrid QM(AM1)/MM potential, the QM part of the structure being shown in Fig. 1. The bulk of the enzyme was kept fixed during the modelling of the hydrolysis reaction, with the positions of the majority of the QM atoms, including the whole substrate being allowed to vary. The QM link atoms, as well as other QM atoms remote from the reactive region, were kept fixed. The AM1 Hamiltonian accurately predicts the energy of proton transfer between isolated imidazole and methanethiol, 132.9 kcal mol^{-1} (1 cal = 4.184 J), compared to the experimental value of 135.0 kcal mol^{-1},[17] thereby giving

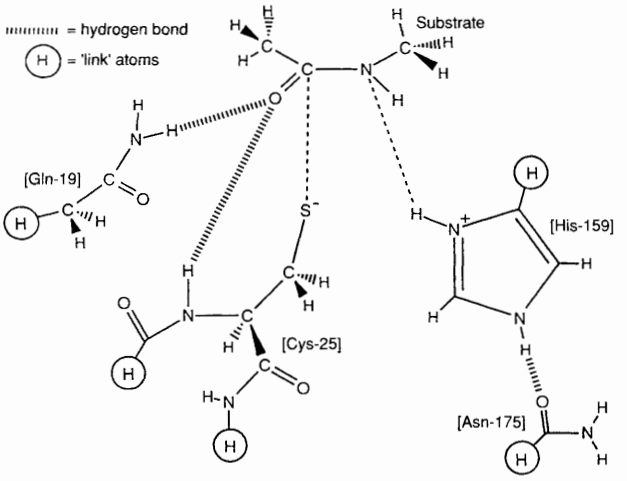

Fig. 1 QM region of substrate–active site

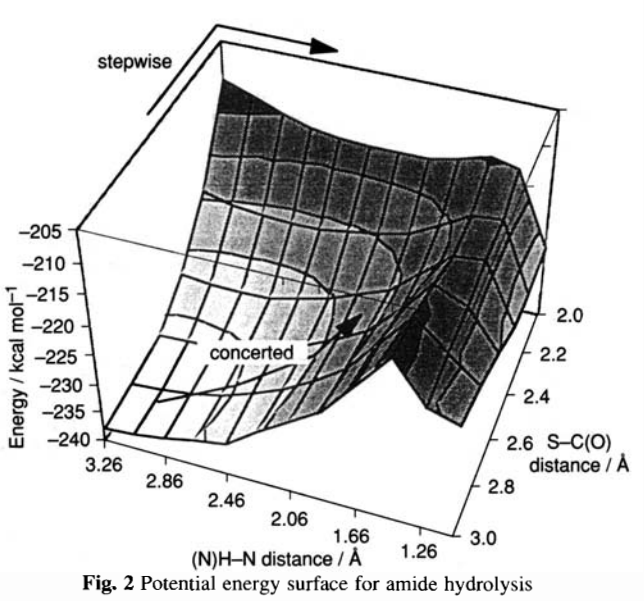

Fig. 2 Potential energy surface for amide hydrolysis

confidence for its use in modelling this macromolecular reaction.

We have mapped out the potential energy surface for amide hydrolysis by minimizing the substrate–active site structure for a range of S^-–C(O) and (N)H–N distances (see Fig. 2) and have conclusively shown that the reaction is a concerted one, proceeding *via* a transition state (Fig. 3) without the intervention of a tetrahedral intermediate. (This structure was subsequently characterized as a transition state by calculation of the harmonic force constants). The predicted structure (Fig. 3) shows that during the reaction, the substrate moves towards the active site increasing the hydrogen bonding with the oxyanion hole (Gln-19, Cys-25) and with the imidazole proton involved in the reaction.

The contribution of Gln-19 to the oxyanion hole which stabilizes the transition state was modelled by repeating the calculation using an enzyme structure in which the residue is replaced by alanine. Such a mutation raises the calculated barrier to the reaction from 20.1 to 21.0 kcal mol^{-1}, close to the experimental increase of 2.4 kcal mol^{-1}.[18] The transition state structure also shows reduced interaction of the substrate with the active site thus reducing its stabilization by the enzyme (Fig. 4). Thus, although these calculations may lack the high accuracy of electronic structure calculations on small molecular systems, we believe that they are of value in predicting the mechanism of enzyme catalysed reactions and identifying transition state structures.

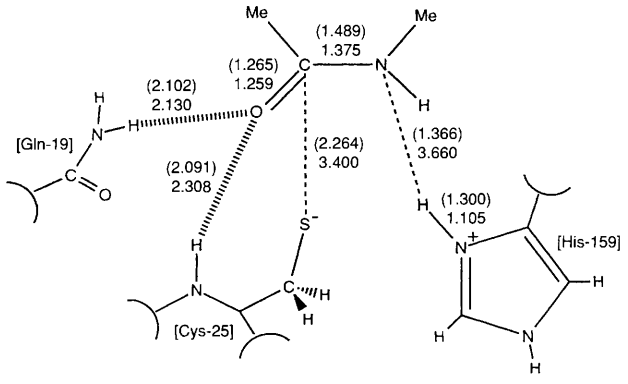

Fig. 3 Active site–substrate geometry. Reactant structure (Å), and in parenthesis, transition state structure.

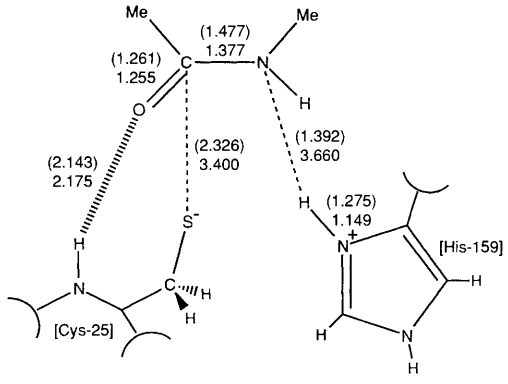

Fig. 4 Active site–substrate geometry with mutation of Gln-19 to Ala. Reactant structure (Å) and, in parenthesis, transition state structure.

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Footnote

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