Triosephosphate isomerase (TIM)-catalysed proton abstraction from carbon acid: an analysis on the origin of the catalytic activity

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Transition-state stabilising hydrogen bonds of the active-site residues and electrostatic solvation energies can explain the major part of the rate enhancement in the triosephosphate isomerase (TIM)-catalysed proton abstraction from carbon acids.

Reaction rates for the enzyme-catalysed proton abstractions of α-protons from carbon acids typically range from 10^1 to 10^4 s^{-1}. The corresponding transition state energies, which range from 55 to 70 kJ mol^{-1}, are *ca* 40–85 kJ mol^{-1} lower than the non-enzymatic counterparts.[1–4] In the case of triosephosphate isomerase (TIM) the energy lowering is 45–50 kJ mol^{-1}. The origin of the rapid rates of the proton abstractions has been a subject of much discussion lately. It has been proposed that the 'low-barrier hydrogen bonds' (LBHB), the strengths of which may be as great as 80 kJ mol^{-1},[5,6] may substantially lower the activation energies.[4,6] However, it has been argued that there is no extra stabilisation by the matched pK_a values[7] and that hydrogen bonds can provide up to 20 kJ mol^{-1} to transition state stabilization.[8] Furthermore, recently Alagona *et al.* showed, that in the case of TIM-catalysed proton abstraction, a model for the substrate enediolate had no intrinsic tendency to accept a proton from an imidazole of the active-site in the presence of the enzyme environment.[9] In this communication we present a computational analysis on the TIM-catalysed proton abstraction. This analysis, which is based on high-level *ab initio* quantum mechanical and electrostatic Poisson-Boltzman calculations, let us conclude that hydrogen bonds of the suitably positioned active-site residues, which need to provide 20–25 mol^{-1} for transition-state stabilisation, and solvation effects can explain the major part of the rate enhancement in the enzyme-catalysed proton abstraction.

Proton abstraction from hydroxyformaldehyde by formate was used as a model for the reaction in the gas phase and in solution. Geometries of **1**, **2**, **3** (TS, 1 imaginary frequency), and **4** (Fig. 1) were optimised at the HF/6-31+G* level using the GAUSSIAN 94 program.[10] Optimised geometries were used in the energy calculations up to the MP4(SDQ)/6-31+G**+ΔZPE (ΔZPE is zero-point vibrational energy difference) level and in estimating the effects of solvent on the relative energies of **1–4**. Polarisable continuum method of Tomasi[11,12] as implemented in GAUSSIAN 94 (IPCM-method)[13] and a value of 0.0004 e B^{-3} for the charge density was applied in the determination of the solute cavity boundary in the solvent calculations.[13] Unscaled frequencies[14] were used in the vibrational analysis and intrinsic reaction coordinate calculations were used to confirm that transition state **3** connects stationary points **2** and **4**. It must be noted that although **3** was characterized as a transition state (HF/6-31+G*) in the gas phase the inclusion of ΔZPE and electron correlation changed the energetic order of **3** and **4**.

As deduced from the development of charge distribution and the geometry of the carbon acid along to reaction coordinate the α-carbon, which loses proton in the reaction, has considerable sp^3 character in **3** indicating that in the transition state the proton abstraction is more developed than resonance in the C=O end of the carbon acid, *i.e.* there is transition state imbalance.[15,16] As compared to the gas phase the inclusion of solvation increases transition-state energy. In water (ε = 78.3) this destabilisation is 12.4 kJ mol^{-1}. After the transition state has been passed the resonance-stabilised enolate anion starts developing (see the C=C distances in Fig. 1) and the C=O end of the carbon acid gaining negative charge. For that reason solvation energy of **4** is 10.3 kJ mol^{-1} more favourable than that of **2** and 22.3 kJ mol^{-1} more favourable than **3**. The inclusion of solvation energies changes the relative order of **3** and **4** as compared to the gas-phase energies. Thus, the computations indicate that proton transfer has an earlier transition state in aqueous solution than in the gas phase. The observation that energy for **1** to **3** increases as ε increases is in line with the experimental results.[15] It has also been experimentally observed that solvents affect rate constants of proton transfers significantly. Part of this is due to change in overall thermodynamics and part due to an effect on intrinsic barrier.[15] The present calculations additionally show that solvent has a larger effect on the complexation step **1** to **2** than the proton abstraction **2** to **3**.

Polar amino-acid residues of the enzyme and solvent affect the energetics of enzyme-catalysed reaction. The contributions of these have been estimated with electrostatic Poisson–Boltzman calculations[17,18] and by using the active-site model of Fig. 2.[19] Dielectric constants used in the Poisson–Boltzman calculations were 2 for the protein interior and 80 for the solvent. Interestingly, the charge distribution of the enzyme had no effect on the relative energies of **2** and **3** while the electrostatic reaction-field energy[17] stabilised **3** by 13 kJ mol^{-1} relative to **2**.[19]

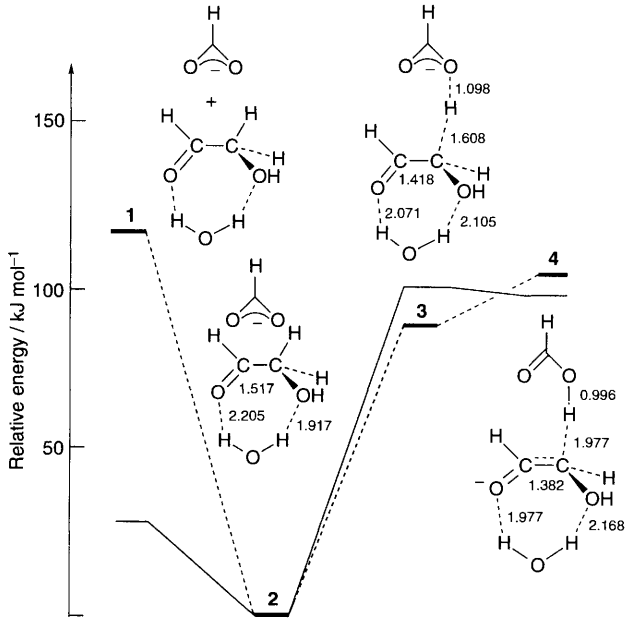

Fig. 1 Structures and relative energies (MP4(SDQ)/6-31+G**//HF/6-31+G* + ΔZPE) of **1–4** in the gas phase (----) and in solution (ε = 78.3, water). Selected hydrogen bond distances are shown (Å). In all the complexes formate makes a hydrogen bond with the OH of the substrate.

The unfavourable solvation energies of steps **1** to **2** and **2** to **3** in aqueous solution probably originates from the fact that formate is desolvated early in the reaction[15] and solvation of enolate, especially because it is not fully developed at the TS, cannot compensate for that. In contrast, it seems that in the partly desolvated active-site of TIM the situation is different and desolvation of the base has much smaller effect on the energetics of the reaction. Complexation **1** to **2** is probably somewhat more favourable in the enzymatic than in non-enzymatic reaction due to the contributions of solvation and entropy on this step. However, because in the case of TIM the encounter of the enzyme and the substrate dihydroxyacetone phosphate (DHAP) is the reaction rate limiting step[20] binding energy is not, at least fully, used to accelerate the enzyme reaction.

In the active site of TIM there are His95, Lys13 and Asn11 residues which form hydrogen bonds with the substrate DHAP (Fig. 2). These residues can accelerate the enzyme reaction by binding more tightly the transition state than substrate. The differential binding energies (**2** *vs.* **3**) of the active-site His95, Lys13 and Asn11 were estimated with a model shown in Fig. 2. Geometries of the species corresponding to **2** and **3** were partially optimized[19] at the HF/3-21G(*) level. In the optimisations the positions of OPO_3^{2-} group of the substrate, Et group of propanoate (Glu167), and imidazole (His95) and acetamide (Asn11) were frozen. In addition, NH_3^+ group of the methylammonium cation (Lys13) was allowed to rotate while positions of carbon and nitrogen were frozen. The active-site models were constructed using the X-ray structure of TIM–glycerol-3-phosphate complex.[21] The interaction energies of the models of His95, Lys13 and Asn11 were calculated by removing them one at a time from the model. Calculated interaction energies for the models of His95, Lys13 and Asn11 were 16, 100 and 8 kJ mol^{-1} (BSSE corrected), respectively, larger in **3** than **2**. Furthermore, when water was replaced by imidazole (model of histidine) in **1–4** (Fig. 1) the relative energy of **3** was lowered by 7.5 kJ mol^{-1} (MP2/6-31+G**//HF/6-31G*) and that of **4** by 13.4 kJ mol^{-1}. Earlier computer simulations of the TIM catalysed reaction have pointed out the importance of Lys13 in accelerating the reaction[22,23] and it has been estimated that His95 lowers transition-state energy by 12.5 kJ mol^{-1}.[22] Further, it has been observed that His95 to Asn mutation increases transition-state energy by 20 kJ mol^{-1} and His95 to Asn, Ser96 to Pro double mutation by 13 kJ mol^{-1}.[24]

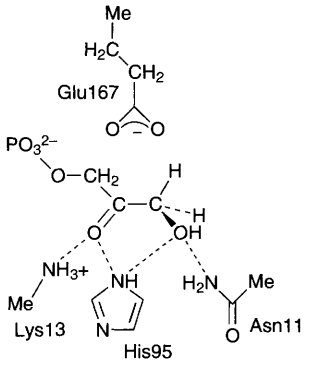

Fig. 2 Active-site model used in the estimation of the contribution of His95, Lys13 and Asn11 on the transition-state stabilisation

This analysis suggest that solvation energies stabilise the proton abstraction within the enzyme by about 25 kJ mol^{-1} as compared to the corresponding reaction in aqueous solution. So, in order to reach the observed transition-state stabilisation of 45–50 kJ mol^{-1} the active-site residues need to contribute 20–25 kJ mol^{-1} to the stabilisation of the enzyme-catalysed reaction. This estimate is in reasonable agreement with the calculated and experimental contributions of the active-site residues. Furthermore, it has been earlier proposed that entropic contribution associated with solvent reorganization in non-enzymatic reactions favours enzyme-catalysed proton abstractions by as much as 25 kJ mol^{-1}.[4] Also, complexation step **1** to **2** is probably more favourable in the enzyme-catalysed reaction than in the non-enzymatic reaction. In conclusion, this work suggest that in the case of TIM the hydrogen bonds between the catalytic amino acids and the substrate contribute no more than 20–25 kJ mol^{-1} to the transition-state stabilisation. This value is considerably smaller than those suggested earlier.[5,6] It must be stressed that more detailed work is needed in order to make conclusions about the degree of protonation of the anionic oxygen of the substrate in the proton transfer.

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