Catalytic Mechanism of Enzymes: Preorganization, Short Strong Hydrogen Bond, and Charge Buffering[†]

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Received January 7, 2002; Revised Manuscript Received February 22, 2002

ABSTRACT: During the past decade, there has been much debate about the enormous catalytic rate enhancement observed in enzymatic reactions involving carbanion intermediates. Our recent theoretical study has demonstrated the importance of the short strong hydrogen bond (SSHB) in the enzymatic reactions. Nevertheless, other recent theoretical studies espouse the role of preorganization over that of the SSHB. To achieve a consensus on this issue and to find the truth, a more clarified explanation must be given. To this end, we have carried out an elaborate analysis of these enzymatic reactions. We here clarify that the catalytic mechanism needs to be explained with three important factors, viz., SSHB, preorganization, and charge buffering/dissipation. Since the charge buffering role is different from the commonly used concepts of the SSHB and preorganization (unless these definitions are expanded), we stress that the charge buffering role of the catalytic residues is an important ingredient of the enzymatic reaction in reducing the level of accumulation of the negative charge on the substrate during the reaction process. This charge reduction is critical to the lowering of activation barriers and the stabilization of intermediates.

The metabolism of living organisms is crucially dependent on enzymatic reactions involving carbanion intermediates (*1*). These intermediates are involved in isomerization, rearrangement, carbon−carbon bond formation and/or cleavage, etc. (*2*). In particular, enzyme-catalyzed allylic rearrangements play a central role in important biochemical pathways. These reactions are also important for chemists in synthesizing novel functional molecules such as drugs and fine chemicals. Therefore, it is of utmost importance to understand the enzyme-catalyzed reaction mechanism. However, it has been a highly controversial issue which revolves around two central concepts of the short strong hydrogen bond (SSHB)[1] (*3*−*14*) and preorganization (*15*−*20*).

The highly debated issue of the role of the SSHB in enzyme catalysis is its H-bond strength. While a direct measurement of the H-bond strength is not possible, the experimental stabilization energies of small model systems are much smaller than the proposed ones (*21*, *22*). Furthermore, the low fractionation factor (*23*) and highly downfield shifted signal in NMR (*24*−*27*) detected in enzyme reactions cannot be directly correlated to the energetic aspects of the SSHB. It was also proposed that the SSHB can be "anti-catalytic" in polar solvents (*15*), and the highly downfield shifted signal in the NMR spectrum need not be a necessary condition for the existence of the SSHB (*28*, *29*). On the other hand, the preorganization concept stresses that the enzyme stabilizes the transition state (TS) over the reactant under suppressed solvent reorganization, and most of this stabilization energy comes from electrostatic interactions (*15*−*18*). Many recent theoretical reports often seem to be against the SSHB concept (*30*−*33*), while some are in favor of it (*34*−*37*). On the other hand, many experimentalists tend to be in favor of the SSHB (*38*−*40*), while some are against it. Recently, we have seen SSHBs in the X-ray structures of ketosteroid isomerase (KSI), which are in agreement with the theoretical prediction (*35*). Furthermore, we have observed short H-bonds (∼2.65 Å) in a one-dimensional H-bond network even in the absence of negatively charged anions (*36*, *37*). We demonstrated the importance of the amphi-acid/base catalytic role of the SSHB and the important contribution of nonelectrostatic energy to the stabilization of TSs and intermediate states (EIs) (*34*). In addition, we stressed that the negative charge dissipates from the substrate to the catalytic residues in the process of enzymatic reaction, thereby lowering the highest occupied molecular orbital (HOMO) energy of the enzyme−substrate system in the TS (*35*). Although this charge buffering effect can be driven by the SSHB, this charge transfer/dissipation/buffering effect is different from that of the SSHB. Thus, we believe that this charge buffering effect needs to be stressed as an additional crucial concept in enzyme mechanisms. Therefore, employing a more elaborate analysis, we explain the catalytic mechanism of enzymes involving allylic rearrangement in terms of three important factors, namely, SSHB, preorganization, and charge buffering/dissipation.

[†] This study was supported by the Creative Research Initiative (KISTEP) of the Korea Ministry of Science and Technology and the Brain Korea 21 project.

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[1] Abbreviations: SSHB, short strong hydrogen bond; TS, transition state; KSI, ketosteroid isomerase; EI, enzyme intermediate state; HOMO, highest occupied molecular orbital; ES, enzyme−substrate; EP, enzyme−product; MO, molecular orbital.

10.1021/bi0255118 CCC: $22.00
Published on Web 03/21/2002

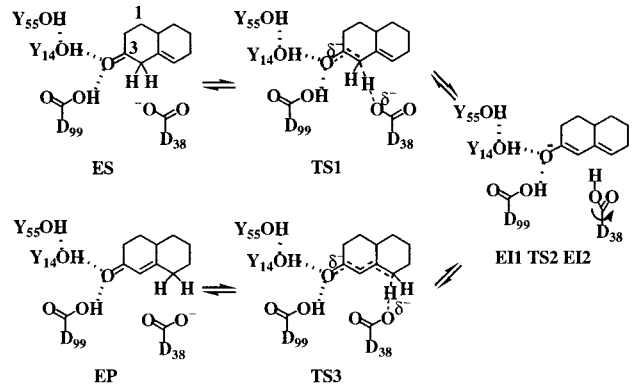


FIGURE 1: Enzymatic reaction scheme of KSI.

THEORETICAL APPROACH

As a paradigm for allylic rearrangement enzymes, we have investigated KSI which exhibits almost diffusion controlled reactivity (*2*, *24*−*27*). The active site of the enzyme has no accessible space for water (*43*−*45*). The key catalytic residues are Asp38, Tyr14, and Asp99 (*24*, *43*−*45*). An ancillary catalytic residue (Tyr55) which is attached to Tyr14 plays the H-bond relay effect, but this effect is not significant compared to those of the three key catalytic residues (*34*, *46*). Since Tyr14 and Asp99 involve H-bonding with the oxyanion (O3), we study three model enzyme systems with (a) none, (b) Tyr14, and (c) Tyr14 and Asp99, while Asp38 is always present in the three cases as an essential residue for a H-shift (from 3−4 to 5−6) in the substrate (steroids). Models b and c can correspond to the experimental systems of the mutant (Asp99Ala) and wild type, respectively. The isomerization reaction (Figure 1) proceeds through seven states: enzyme−substrate state (ES), abstraction of the C4 β-proton (TS1), first enzyme−intermediate state (EI1), rotation of protonated Asp38 (TS2), second enzyme−intermediate state (EI2), proton donation to the C6 β-position by Asp38 (TS3), and enzyme−product state (EP). The reaction is composed of three elementary reactions: (1) abstraction of the C4 β-proton by Asp38 (ES → TS1 → EI1), (2) slight rotation of protonated Asp38 (EI1 → TS2 → EI2), and (3) proton donation to the C6 β-position by Asp38 (EI2 → TS3 → EP); the initial (for the reactant) and final (for the product) elementary reactions due to the diffusion in solution are not considered here.

Previously, using ab initio calculations, we explored the structures and energies of the systems for the KSI enzymatic reaction steps along the isomerization reaction path (*34*). The calculated results (structure, chemical shift, and reactivity) were in good agreement with the experimental data (X-ray, NMR, and kinetics data). To facilitate our discussion, the energy profiles for model cases a−c which were obtained using the density functional theory calculations with three Becke parameters employing the Lee−Yang−Parr functional and 6-31G* basis set (B3LYP/6-31+G*) (*34*) are depicted in Figure 2. Whenever necessary, we added more reliable Moller−Plesset second-order perturbation (MP2) calculations at the B3LYP/6-31+G* geometry (MP2/6-31+G*/B3LYP/6-31+G*). Here, we analyze the enzymatic mechanism based on the refinement of the previous calculations that included self-consistent reaction field (SCRF) analysis for the medium effect (*35*) for the clarification of the enzymatic role, since the issue of the SSHB versus preorganization has been so controversial.

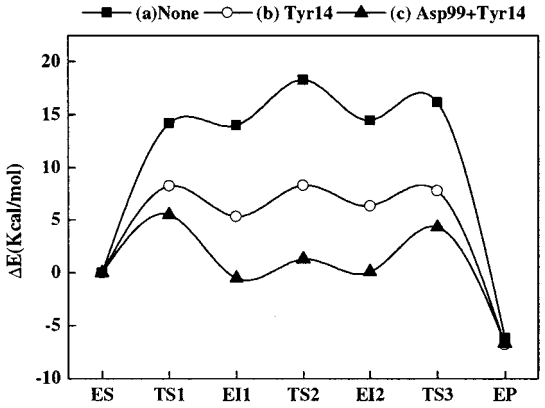


FIGURE 2: Calculated energy profiles along the reaction path for cases a (none), b (Tyr14), and c (Tyr14 and Asp99) (at the B3LYP/6-31+G* level). More reliable MP2/6-31+G*/B3LYP/6-31+G* results increase the activation barriers by ∼20%.

RESULTS AND DISCUSSION

According to the preorganization concept, the preorganized dipoles of the enzyme are expected to stabilize the TS over the reactant under suppressed solvent reorganization. The stabilization is predominantly due to electrostatic interactions between catalytic residues and the substrate. Recently, we reported that a large portion of the stabilization energy (5−10 kcal/mol) by catalytic residues is electrostatic, since the enhanced H-bond energy at TSs with respect to the normal H-bond (*47*, *48*) at ES arises from the charged H-bond (*49*), as H-bonds between neutral partners (at ES and EP) are very different from H-bonds involving ionic species (at EIs and TSs). However, this energy gain would be better explained in terms of the SSHB concept instead of the preorganization concept. Since the orientations of −OH (or −NH) groups are highly flexible to have the optimal interactions for both ES and TS states and the H-atoms in the H-bonds can be transferred to the other anionic site (i.e., substrate/residue), the −OH (or −NH) group cannot be simply considered a preorganized dipole. Nevertheless, both H-bonds and charged H-bonds form similar orientations, and so the H-bond orientations do not tend to change severely during the reaction process. Thus, a major portion of the electrostatic energy gained by the charged H-bonds in SSHBs is related to that gained as a result of the preorganization.

Furthermore, although normal H-bond energies which are mostly electrostatic are practically additive, we note that the stabilization energy by two catalytic residues is subadditive. At TS1, the predicted stabilization energy caused by the presence of either Asp99 or Tyr14 with respect to case a (none) is 6.1 or 6.3 kcal/mol, while that caused in case c (Tyr14 and Asp99) is 10.1 kcal/mol in contrast to the additive sum (12.4 kcal/mol). In the presence of two catalytic residues, the charge of the oxyanion is redistributed to H-bonds, which causes the subadditive stabilization of the intermediate as a result of the reduced proton-withdrawing power of the oxyanion by each residue due to the presence of the other residue. Thus, it indicates that the H-bonds would involve nonelectrostatic interactions such as charge transfer, charge dissipation, polarization, dispersion, etc. (*46*). Indeed, the negative charge of the substrate is much reduced in the

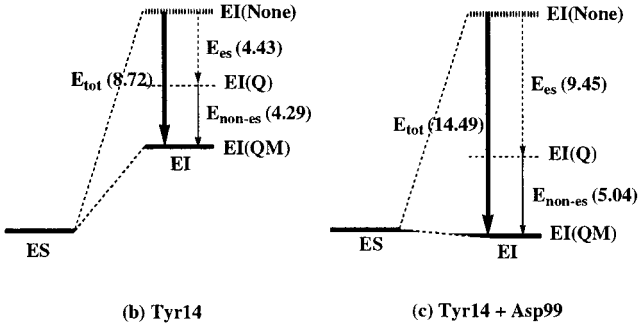


FIGURE 3: Decomposition of the stabilization energy (E_{tot}) for the intermediate state (EI) into electrostatic (E_{es}) and nonelectrostatic (E_{non-es}) terms on the basis of the B3LYP/6-31+G* calculations. For the MP2/6-31+G**//B3LYP/6-31+G** calculations of cases b and c, the E_{tot} values are 9.7 and 17.8 kcal/mol, respectively; the E_{es} values are 5.0 and 11.9 kcal/mol, respectively, while the nonelectrostatic energies (E_{non-es} which corresponds to the non-preorganization energy) are 4.7 and 5.9 kcal/mol, respectively.

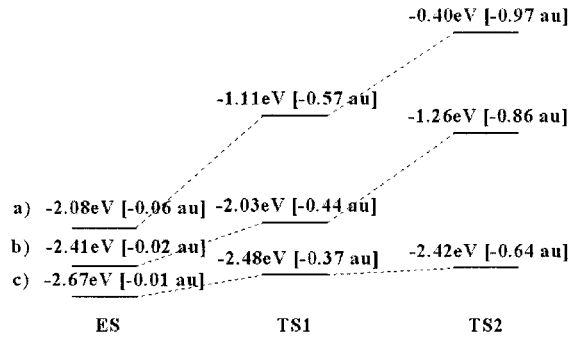
presence of two catalytic residues; the effective natural bond orbital population charges of the substrate (q_s) in cases a−c at TS1 are −0.57, −0.44, and −0.37 au, respectively.

To better understand the nature of the binding between catalytic residues and the substrate, we have investigated the contribution of electrostatic interaction (E_{es}) to the stabilization energy (E_{tot}) of the EI1 intermediates using our previous calculational strategy (*34*). To evaluate the E_{es}, we carried out the calculations for the ES and EI1 in the case when catalytic residues are replaced by ghost residues composed of only their charges which were calculated for each isolated catalytic residue. The contribution of nonelectrostatic interaction (E_{non-es}) is the difference between E_{tot} and E_{es}. The predicted energy decomposition is depicted in Figure 3. For the MP2/6-31+G**//B3LYP/6-31+G** (B3LYP/6-31+G*) calculations of cases b and c, the E_{tot} values are 9.7 (8.7) and 17.8 (14.5) kcal/mol, respectively; the E_{es} values (based only on the charges of the ghost residues) are 5.0 (4.4) and 11.9 (9.5) kcal/mol, respectively, while the nonelectrostatic energies (E_{non-es} which corresponds to the non-preorganization energy) are 4.7 (4.3) and 5.9 (5.0) kcal/mol, respectively. These results show that while the electrostatic interaction term of the SSHB driven by preorganization is very important in the enzymatic reaction, the energy gain by nonelectrostatic terms (charge transfer, charge dissipation, polarization, etc.) is also important and can be somewhat comparable to the (noninduced) electrostatic energy gain. However, the molecular orbital (MO) analysis indicates that the nonelectrostatic energy does not arise from the covalent character of the SSHB.

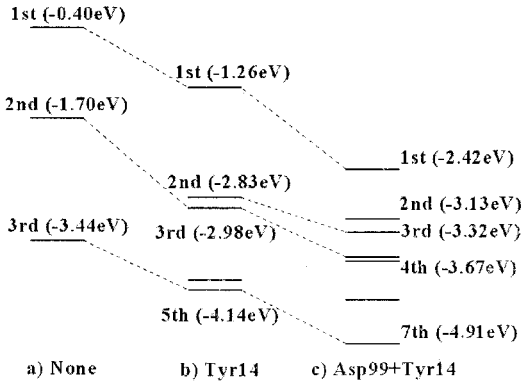
A Nonelectrostatic Nature Comes from Charge Dissipation. Here, we report that the nonelectrostatic interaction arises mainly from MO interaction energy due to charge dissipation. The catalytic residue plays the role of charge buffer which keeps a portion of the negative charge accumulated on the substrate during the reaction process (at TSs and EIs). As shown in the reaction scheme (Figure 1), the negative charge of Asp38 migrates to the substrate at TS1, resulting in delocalization/dissipation of the charge toward substrate O3, the π-conjugated bond between positions 3 and 4, and the partial π-conjugated bond between positions 5 and 6. As we already mentioned that the charges of the substrate in cases a−c at TS1 are −0.57, −0.44, and −0.37 au, respectively, the negative charge of the substrate accumulated at TSs and EIs is much reduced by the catalytic residues which play the charge buffer role. This reduction arises from the transfer of the excess electron from the substrate to the catalytic residues, resulting in the lowering of the energy of the excess electron related to the HOMO state, as the space to accommodate the excess electron is enlarged with catalytic residues in addition to the substrate. This energy lowering is directly related to the uncertainty principle; the less localized the excess electron, the more stabilized it is (*51*), since the excess electron at TSs is much loosely bound than that at ES.

We have investigated the orbital energy levels and electron density maps (Figure 4) of valence orbitals at ES and TSs. At ES, TS1, and TS2 of case a (none), the HOMO energies are −2.08, −1.11, and −0.40 eV, respectively, with their respective substrate charges (q_s) of −0.06, −0.57, and −0.97 au (Figure 4A). In case b, because of the charge buffering role of Tyr14 which reduces the negative charge accumulated at TSs, the above three HOMO energies are −2.41, −2.03, and −1.26 eV, respectively, with respective q_s values of −0.02, −0.44, and −0.86 au. This charge buffering role is further enhanced by two catalytic residues in case c, and so the HOMO energies of the above three states are −2.67, −2.48, and −2.42 eV, respectively, with respective q_s values of −0.01, −0.37, and −0.64 au. In this case, the HOMO energies are somewhat constant along the reaction path. The HOMO energy lowering due to the reduction of extra negative charge to be accumulated in the substrate at TSs should be responsible for the strong stabilization of the system along the reaction path, i.e., for the drastic lowering of the activation barrier, and hence for the enormous increase in reactivity. Panels B and C of Figure 4 show how the negative charge of the substrate is greatly reduced. They show that a portion of the negative charge density of the substrate is stored in catalytic residues (Asp99 and/or Tyr14 which act as a charge buffer) as second and/or third HOMOs (Figure 4C) at TS2. Consequently, the MO interaction comes from electron rearrangements associated with charge dissipation from the substrate to catalytic residues. The charge dissipation delocalizes the excess electron over the large space spanning both the substrate and catalytic residues.

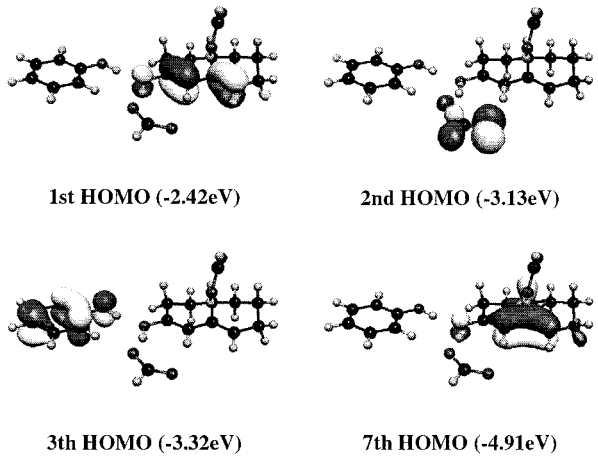
Figure 5 shows how the frontier MOs are constructed for ES and EI1 in case b, Tyr14 from their substrate and residues. The neutral charge state of case a is defined as SA (=substrate + Asp38), and the negatively charged state [a (none)] is represented as SA^-. The neutral states of SA and Tyr14 have low-valence orbital energies. When these are negatively charged with an excess electron, the valence orbital energies become high. Because of the charge dissipation from the substrate to residues, the excess electron delocalized over the space spanning both the substrate and residues behaves not only as an excess electron of SA but also as an excess electron of Tyr14, i.e., a dual role of a HOMO of SA^- and a HOMO of $Tyr14^-$. Consequently, the valence orbital energies for case b are similar to those of the combined system with SA^- and $Tyr14^-$ instead of SA^- and Tyr14 or SA and $Tyr14^-$. Of course, the valence orbital energies of case b, i.e., $[(SA \text{ and } Tyr14)^-]$, are slightly lower than the corresponding ones of the combined system (SA^- and $Tyr14^-$) due to the absence of the second extra negative charge. At ES of case b, the valence orbital energies of (SA



[A] ES/TS1/TS2 : 1st HOMO [q_s]



[B] TS2 : Valence Orbitals (HOMOs)



1st HOMO (-2.42eV) **2nd HOMO (-3.13eV)**

3th HOMO (-3.32eV) **7th HOMO (-4.91eV)**

[C] TS2 : (c) Asp99+Tyr14

FIGURE 4: HOMO energies (in parentheses) and charges (in brackets) at the reactant state (ES) and transition states (TS1 and TS2) of three model enzymes: (a) none, (b) Tyr14, and (c) Tyr14 and Asp99. [A] Valence orbital energies at TS2 of cases a−c. [B] Electron density maps at TS2 of case c. [C] B3LYP/6-31+G* level.

and Tyr14)$^-$ are more similar to those of SA$^-$ than Tyr14$^-$, while at EI1, they are more similar to those of Tyr14$^-$ than to those of SA$^-$. This indicates that as the reaction proceeds from ES to EI1, a portion of negative charge to be accumulated on the substrate transfers to Tyr14, resulting in significant accumulation of negative charge on Tyr14 at EI1. This results in a small amount of HOMO energy elevation in the system (from −2.41 eV at ES to −1.47 eV at EI1), which contrasts with the case without a catalytic residue (i.e., case a or SA$^-$) involving a large amount of HOMO energy elevation (from −2.08 eV at ES to −0.81 eV at EI1). The effect of two catalytic residues in case c (Tyr14 and Asp99) is much greater. It is, therefore, clear how the charge dissipation lowers the HOMO energies or how the EIs and TSs are stabilized in the presence of catalytic residues.

If the catalytic residue is substituted with nonpolar aprotic groups (Leu and Phe in this study, which can hardly form H-bonds with the substrate), the stabilization energy by charge dissipation/buffering from the substrate to the residues is estimated to be 3.8 kcal/mol at TS1 and 3.2 kcal/mol at EI1 (Figure 6). Therefore, it should be stated that even nonpolar residues play a significant role in an active site wherein the reorganization of residues is suppressed. On the other hand, if the substrate is in a polar medium ($\epsilon = 18$) (*26*), the TSs and EIs are slightly destabilized, which shows an anticatalytic effect, as already addressed in earlier works (*15*). Thus, we note that the charge dissipation from the substrate to residues is quite important for the catalytic role in stabilizing TSs and EIs, while the localized charges and dipole moments due to the dielectric medium do not play a significant catalytic role.

Amphoteric Role of an Enzyme through the SSHB. It should be stressed that the most critical role of SSHBs in enzymatic reactions is the dual role as amphi-acid/base catalysts, which is responsible for the low activation barrier. In the reaction pathway of KSI, there are two rate-determining steps: abstraction of the C4 β-proton by Asp38 and reprotonation to the C6 β-position by protonated Asp38 (Figure 7). The enolate intermediate is stabilized by the SSHB from Asp99 and Tyr14. In the first step of the reaction, Asp38 functions as the nucleophile (general base) and Asp99 and Tyr14 act as the general acid (at the same time). In the second step of the reaction, all the catalytic residues (Asp38, Asp99, and Tyr14) play roles opposite from those of the first step. We denote Asp38 as R1, and Tyr14 and Asp99 as R2, to distinguish two residue groups in that R1 and R2 play the opposite roles during the reaction. In each step, the catalytic residue groups (R1 and R2) contribute to the lowering of the activation barrier by the concerted action of acid and base. When the entire reaction is considered, the strong general acid catalyst R2 stabilizes TS1/EI1 in the first step in terms of activation energy, but destabilizes TS3/EI2 in the second step, since the general acid (HA) should donate a proton to the substrate as R1 abstracts the C4 β-proton. On the other hand, the strong general base catalyst R2 is just the opposite. Thus, for KSI to have a low barrier height at each TS step, an acid catalyst of R2 is used at TS1, while a base catalyst of R2 is used at TS3. This criterion can be met if the general acid (HA) in TS1/EI1 should play a general base (A$^-$) as a conjugate in TS3/EI2. In general, it would not be feasible for a well-preorganized enzyme to show completely opposite catalytic behaviors at the same active site without SSHBs. In KSI, the SSHBs between the residues and the oxyanion play the acid catalytic role at TS1, and the base catalytic role at TS3. Thus, in the two-step reaction mechanism of KSI, the SSHBs play a crucial dual role as amphi-acid/base catalysts along with partial proton shuttles and charge redistributions (or electron rearrangements). This is the reason SSHBs are often found in enzyme catalysis, which is consistent with the proposal of the role of SSHBs in enzymatics involving carbanions.

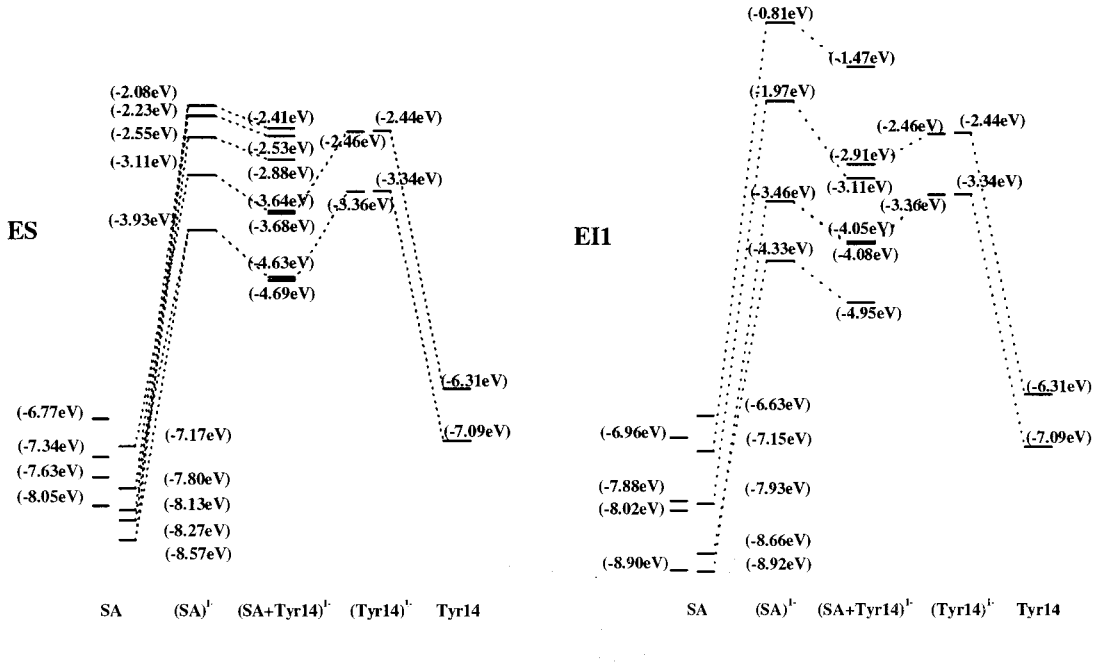


FIGURE 5: Comparison of valence orbital energy levels at the reactant (ES) and intermediate (EI1) states of SA, SA$^-$, (SA and Tyr14)$^-$, Tyr14$^-$, and Tyr14 (B3LYP/6-31+G* level). SA (=steroid + Asp38) denotes the neutral charge state of the combined system of the steroid (substrate) and Asp38, which corresponds to case a (none), while (SA+Tyr14)$^-$ corresponds to case b (Tyr14). The singly occupied orbital of Tyr14$^-$ (2.38 eV), which is not drawn in the figure, corresponds to the lowest unoccupied MO (LUMO) of (SA+Tyr14)$^-$ (1.84 eV at ES and 2.32 eV at EI1).

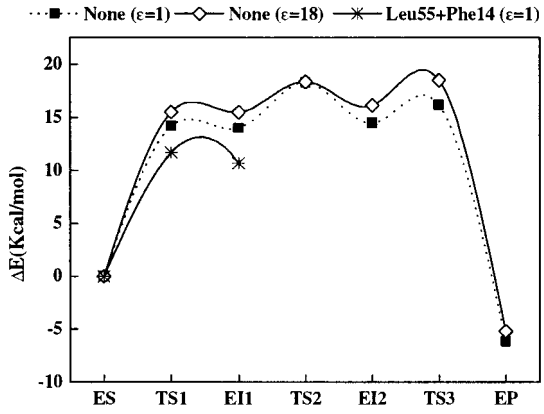


FIGURE 6: Energy profiles along the reaction path for case a (none) where the substrate is in a nonpolar medium ($\epsilon = 1$), case a′ where the substrate is in a dielectric medium ($\epsilon = 18$), and case a″ where the substrate interacts with Leu and Phe in a nonpolar medium ($\epsilon = 1$) (SCRF calculation at the B3LYP/6-31+G* level).

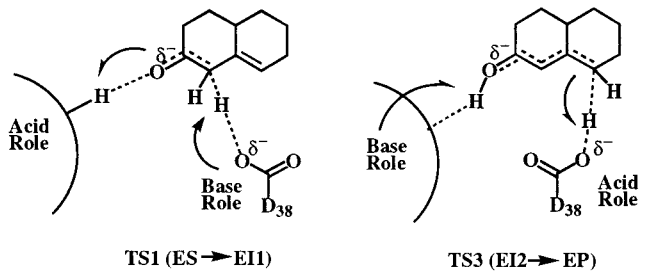


FIGURE 7: Catalytic strategy for stabilizing the transition state via an amphoteric role through the SSHB.

The best condition for stabilizing the two rate-limiting reaction steps would be the case when both barriers of proton transfer between ES and EI1 and between EI2 and EP are low. As discussed earlier, the case of either general acid or general base catalysis would favor only one of the two rate-limiting steps in KSI, while the other step is disfavored. In the two-step reaction mechanism, if the HA has the same proton affinity (p*A*) with the substrate at TSs/EIs, the catalytic activity tends to be optimized. Thus, the SSHBs with matching p*A* values between two interacting sites are well suited. It should be noted that in KSI, the catalytic residues are surrounded by apolar residues but not by water, and so the pK_a of the residues in the enzyme would be more similar to the p*A* values in the gas phase than the pK_a in bulk water, as seen in the experiments. [The pK_a of Asp99 in the active site of the enzyme is ~9, while that in bulk water is only ~4 (*44*); the pK_a value of a catalytic residue can change greatly during catalysis (*52*, *53*).] Thus, in the presence of two catalytic residues, the matched p*A* indicates the match between the p*A* of the substrate and the combined p*A* of the two residues. We would like to stress that *in two-step enzymatic reactions* SSHBs play dual roles as general acid and general base catalysts, resulting in two low activation barriers. However, it should be noted that *in one-step reactions*, either the general acid or general base catalyst should play a much stronger catalytic role over the SSHB, leading to a lower activation barrier.

Remarks on the SSHB, Preorganization, and Charge Buffering. We have already stated that the SSHB concept has at times been refuted, while some still favor the SSHB concept. Very recently, a number of authors have espoused the case of preorganization over that of the SSHB in stabilizing the TS. Thus, we need to clarify whether the preorganization and SSHB are incompatible. First, the energy gain by the low barrier for proton transfer is clearly small. For example, the H-bond energy does not significantly

increase (by no more than ~1 kcal/mol in KSI) after the proton is allowed to relax. The energy difference between the case when the OH bond length is fixed and the case when the H atom is fully relaxed is not significant. For this reason, the low-barrier H-bond (LBHB) concept has at present been extended to mean a more general term of the SSHB. It is clear that the SSHB exists in the gas phase, as in $F\cdots H\cdots F^-$ (*54*). In enzymes, many X-ray structures have shown the existence of short H-bonds (<2.6 Å) (*35*, *43*, *44*). Nevertheless, it is possible that in a polar solution, SSHBs can be anticatalytic. However, the active sites of enzymes are not easily randomized because the residues are well-preoriented. In this way, it is natural to accept the importance of preorganization for which the entropy increase by the randomization of the residue configuration is suppressed and the preoriented dipole orientations of the polar residues in an active site are electrostatically in favor of the TS rather than the ES. However, this preorganization concept also stresses that electrostatic energy dictates everything in enzyme reactions, and as a result, the energy gain by structural changes during the reaction process is not seriously considered. Thus, the preorganization energy includes some electrostatic energy gains by SSHBs over normal H-bonds (i.e., electrostatic energy gains by charged H-bonds), but it excludes some electrostatic energy gains by SSHBs due to the structural changes of the substrate and residues [i.e., the flexible orientations of the −OH (or −NH) groups and the possible transfer of the proton between two anionic sites involving a charged H-bond]. In addition, the preorganization concept excludes the nonelectrostatic energy gain by the SSHB, while this term is minimal because the covalent character is negligible in the SSHB. On the other hand, the residue-driven nonelectrostatic energy gain by charge dissipation is important. Although this energy gain does not belong to the SSHB energy gain, it can be said to be SSHB-driven induction energy, because charge dissipation of the anionic substrate arises from SSHB to stabilize the oxyanion and induce the changes in bond orders in the substrate. The charge dissipation from the substrate to residues can be described by neither the SSHB nor preorganization, unless these concepts are extended. The SSHB concept has enhanced H-bonding energy which is clear from the H-bond being shorter than the normal H-bond. This shortening was clearly demonstrated in the X-ray structures (*35*). Furthermore, this shortening clearly explains the enhancement in bond energy on the basis of the uncertainty principle. Thus, the SSHB can be explained neither by preorganization nor by charge buffering. On the other hand, the preorganization concept has an entropy energy gain by the suppressed solvent reorganization that neither the SSHB nor charge buffering can have.

In explaining the catalytic activity by enzymes, we describe the clear distinction between preorganization, charge dissipation, and the SSHB as followes. (i) Preorganization requires almost the whole enzyme for electrostatic energy gain mostly due to permanent polarization (i.e., noninduced polarization) without entropic energy loss due to reorientation of the permanent dipole charges in enzyme residues, unlike in solvents. (ii) Charge buffering requires only key residues for charge dissipation to be responsible for almost all induced polarization. (iii) The SSHB focuses on the enhanced strength of the H-bond. It should again be stressed that charge buffering is responsible for the lowering of the activation barriers and stabilization of intermediate states because of the dissipation of the negative charge of a substrate (in TSs and intermediate states) to the catalytic residues (which play the buffer role by holding a portion of extra negative charge). Therefore, neither preorganization nor the SSHB includes the induced polarization which stabilizes the TSs and intermediates of a substrate. As the concepts of these terms are extended, preorganization would include the induced polarization, and the SSHB would include the SSHB-induced polarization. However, since all three concepts can be more clearly defined as they are, the three important factors are essential in explaining the catalytic rate enhancement observed in enzymatic reactions involving carbanion intermediates.

CONCLUDING REMARKS

We analyzed the enzyme mechanism based on our previous ab initio results of KSI which were in good agreement with various experimental data. The origin of the fast diffusion reactivity or low activation barrier in the catalytic mechanism is described in terms of preorganization, the SSHB, and the charge buffering/dissipation role of catalytic residues through the SSHB. Large stabilization energy for EIs/TSs relative to ES comes from (i) the enhanced H-bond energy (of charged H-bonds relative to the normal H-bond), (ii) the MO interaction energy (by the electronic charge redistribution due to charge transfer and polarization involving excess electron dissipation to the catalytic residues), (iii) amphi-acid/base role of the SSHB, and (iv) suppression of the entropy increase by suppressed solvent reorganization. The contributions from parts i and iii are described by the extended concept of the SSHB, while the contribution from part iv and a portion of the contribution from part i are described by preorganization. Here, a portion of the energy gain in part i is described by preorganization as well as the SSHB. An important point of this study is to stress the charge dissipation/transfer/buffering effect in enzymatic reaction, which can be described by neither the preorganization concept nor the present SSHB concept. Charge buffering is responsible for the lowering of the activation barriers and stabilization of intermediate states because of the dissipation of the negative charge of a substrate (in TSs and intermediate states) to the catalytic residues (which play the buffer role by holding a portion of extra negative charge). Finally, we should stress that the SSHB, preorganization, and catalytic residue-driven charge dissipation/buffering are all important in two-step enzymatic reactions involving carbanion intermediates.

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BI0255118