Effects of Donors and Acceptors on the Energetics and Mechanism of Proton, Hydrogen, and Hydride Release from Imidazole

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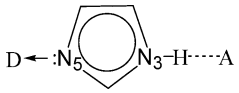
The mechanism and energetics of H-unit (proton, hydrogen, hydride) release from the N−H site of imidazole (IM) is explored on the basis of B3LYP/6-311++G** calculations. Of the three H-unit release modes of IM, H-atom release is strongly favored in the gas phase. Protonation of the bare N-site, positive charge injection over the whole IM ring, and water coupled to the N−H site can each significantly decrease the proton dissociation energy but separately do not change the relative release priority of the proton and H-atom. Their cooperative effect not only significantly decreases the proton dissociation energy but also makes proton release more favorable than H-atom release. A point charge of variable-magnitude placed at the bare N-site leads to a crossover between proton and H-atom dissociation to favor proton release around 1.8 charge units. When a hydrogen-bonded water is added to the N−H site, the crossover that favors proton release falls to a much smaller value (∼0.4 charge units). Thus, proton release, which is dominant in many normal biological processes, may result from a combination of positive charge or positively-charged particle catalysis at the bare N-site and a basic species assisting at the N−H site.

I. Introduction

The investigation of proton dynamics continues as one of the most active subjects owing to its importance in enzymatic reaction mechanisms[1] and proton transport pathways (translocation) through membrane proteins.[2] The mechanistic details and kinetic properties of proton transfer and translocation in proteins are strongly influenced by the environment of the proton donor and acceptor sites. Imidazole (henceforth denoted as IM), an essential constituent of many biological compounds such as adenine, guanine, histamine, and the histidine residue of proteins, exhibits particularly versatile chemistry[3] since it, and its derivatives, form a class of nucleophilic and general base catalysts.[1] In its incarnation as the functional group of the histidine residue with a pK_a of 6.5, IM is commonly associated with protein residues and cofactors that act to transport protons from one site to another site using the donor and acceptor properties of IM and protonated IM. The increasing ability to experimentally determine the protonation state of protein residues, in particular histidines, makes it attractive to try to find rules that will determine the ionization state and hydrogen bonding pattern of histidine.[4,5]

Many recent experimental and theoretical works have focused on the structural[6−10] and energetic properties of IM in its free and bound state[11−16] including its protonation and deprotonation energies. The calculated protonation energy of IM at the N5-site (see Scheme 1) is 231.6 ∼ 238.5 kcal/mol[12,17] and the deprotonation energy of IM is 346 ∼ 369 kcal/mol.[11,14] Since the deprotonation energy of protonated IM is far smaller than that of the free IM, the cationization of the bare N-site can significantly decrease the dissociation energy (De) of the N−H site proton. Our preliminary calculations found that the N−H site of IM may actually release a hydrogen atom (H) (De ∼ 120 kcal/mol) with a large preference relative to H^+ to H^-,

SCHEME 1



subject to the specific surroundings. Comparison of this value with the proton De of IM shows that the H-unit released from the N−H site of the IM ring should be an H-atom instead of a proton for both free IM and the bare N-site protonated IM. Even if a cation such as Zn^{2+} is coordinated to the bare N-site, H-atom release (De ∼ 120 kcal/mol) is also still competitive with proton release (De 117 ∼ 121 kcal/mol).[14] These observations, based on the gas phase, differ from those in typical condensed phases such as proteins.

It is worth noting that proton and H-atom release should be classified as two distinct processes, because the latter includes a "cooperative" transfer process of a proton with an electron. Indeed, proton transfer may be followed by electron transfer (proton-coupled electron transfer) or cooperatively as H-atom transfer, and the operative mechanism is determined by the surroundings. There are numerous experimental and theoretical explorations in biological systems of proton-coupled electron transfer and hydrogen or hydride transfer.[17−26]

In this work we address by computation the issue of what (minimal) surroundings of IM will determine the preference for H-unit release as H^+ rather than H or H^- release. In general, it is recognized that solvation[27] and, more relevant to biological applications, specific solvation effects, catalyze H-unit transfer. Because there is such a variety of specific effects possible, we adopt a strategy of introducing different charges and charge distributions into imidazole. In this manner we may investigate the dependence of the H-unit release energetics on surrounding factors such as the charge and character of the attacking groups to the bare N-site of the IM ring. Essentially, the transferred or released H-unit may be a proton with a different amount of electrons (in the range from 0 to 2), depending on the

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surroundings. Especially for H-atom or hydride anion release, large electronic redistributions and significant structural changes in the donors and acceptors may result. The following charge introduction methods will be used. 1) Charge attachment with H^-, H, and H^+ (equivalent to the imidazolate cation). 2) Charge injection of a unit + and − charge that is distributed over IM. 3) A point charge scan method where a positive point charge of variable magnitude is attached to the bare N: of IM. These three methods will also be used with hydrated versions of these species where one or two waters are hydrogen bonded to the N3 and N5−H sites of IM in various permutations. In this fashion, we can build up a picture with some generality of the types of charge distributions of IM and hydrated IM that will favor proton release that is dominant in proteins.

There are numerous examples of histidine participating in proton-transfer reactions that may be summarized by Scheme 1 where there is a donor (D) interacting with the N5 nitrogen and an acceptor (A) interacting with the N3−H moiety. Clearly, imidazole is not equivalent to a histidine residue; in particular, the two nitrogens of histidine are not equivalent and there are some propensity rules for which nitrogen carries the hydrogen in neutral histidine.[4,5] Such refinements are beyond the considerations herein. A few noteworthy systems that can be formulated as in Scheme 1 follow:

1) The photosystem II oxygen-evolving complex has a tyrosine (denoted as Y_Z), acting as the donor (cf. Scheme 1); that is, hydrogen bonded to a histidine in the catalytic, manganese-containing site.[28−33] In a particular reaction step, the N5 site of the histidine is the initial acceptor of the phenol proton released upon the Y_Z tyrosine's oxidation by the reaction center chlorophyll complex P_{680}.[34] Once the histidine is protonated, the N3−H deprotonates to a chain of hydrogen bonded waters and residues, the acceptor, ultimately connecting to the bulk. Qualitatively, after Y_Z oxidation, the phenolic proton is much more acidic and the effect on the histidine can be modeled either as charge injection over the histidine or with a point charge of some magnitude at the N5 site.

2) As well appreciated, histidine is central to the catalytic mechanism of peroxidases, which reduce peroxides to alcohols.[1] The substrate may be small such as H_2O_2 in cytcochrome *c* peroxidase or large, as in prostaglandin endoperoxide H synthases (PGHSs), where a fatty acid endoperoxide/hydroperoxide substrate is used. The "push−pull"[35,36] mechanism of peroxidase catalysis invokes a proximal histidine that is ligated to the heme iron (the donor) of a porphyrin through its bare N5 site as well as a histidine on the distal side of the heme that is used for heterolytic cleavage of the oxygen−oxygen bond of the substrate. Evidence for tautomerization of the proximal histidine N3−H proton to a neighboring acceptor such as an aspartate has been found by Resonance Raman studies.[37,38] This tautomerization is driven, in part, by the charge distribution experienced by the N5 site. In PGHSs, the peroxidase site activity involves binding of the *15-hydroperoxyl* group of PGG_2 to the heme iron, with concomitant donation of a proton to a distal histidine from the peroxyl group coordinated to the heme iron. Subsequently, there is proton transfer from this distal histidine to the other peroxyl oxygen that results in an acid−base-catalyzed heterolytic cleavage of the oxygen−oxygen bond. These protonation/deprotonation events are strongly influenced by the evolving charge distributions of the proximal and distal histidines' imidazole.

3) The Zn^{2+} cation is a cofactor in many enzymes that plays either a structural or catalytic role.[39−41] The coordination often is tetrahedral and typically involves one or two histidines. The Zn^{2+} is the donor to the N5 site and there are various acceptors, from water(s) to nominally-ionized residues such as glutamate or aspartate. When the acceptor is acetate, recent calculations have shown that the Zn^{2+} donor can render the ligated histidine sufficiently acidic to produce imidazolate-acetic acid (versus imidazole-acetate).[42] That histidines will be in their histidinate form has been found in numerous protein active sites.[40,41]

That it is also of interest to examine all modes of imidazole H-unit release can be appreciated from the following considerations. In solution, it is no surprise that the major H-unit release mode of IM is as a proton since the solvation energy of the proton is so large, approximately 260 kcal/mol.[27] However, in the much less polarizable interior of many enzyme active sites it is not so clear that proton release should be dominant. Indeed, in metalloproteins some amino acid ligands routinely participate as radicals as part of electron/radical/proton-transfer pathways. For examples, tyrosine radicals participate in redox pathways in ribonuclease reductase,[43,44] prostaglandin endoperoxide synthase[45] and photosystem II[46] and tryptophan radicals are important in DNA photolyase[47] and cytochrome *c* peroxidase.[48] From electronic structure considerations one might anticipate that histidine would also be a good candidate for radical transfer pathways. Yet, there is scant experimental evidence for histidine radicals in proteins. As noted before,[49] this may be linked to the fact that EPR measurements would exhibit large line broadening when a radical is in close proximity to a paramagnetic metal site. Thus, the lack of evidence for histidine radicals may be an issue of detection rather than their absence from the protein.

The remainder of this paper is structured as follows: Section II briefly describes the computational method that is used. Then, the analysis of the H-unit release mechanism and the corresponding energetics and various factors that contribute to the properties of the released H-units are presented in Section III. Our concluding remarks are given in Section IV.

II. Computational Methods

All calculations are performed using B3LYP density functional theory method with a 6-311++G** basis set that includes polarization and diffuse functions on both hydrogen and all heavy atoms, to describe the behavior of a proton or hydrogen atom. To confirm the accuracy of the relevant calculated quantities, the structures, frequencies, proton affinity, and deprotonation energy of the N−H site of the well studied species, the isolated IM molecule, for which all these parameters are available experimentally, are first determined by using several different theoretical methods with different basis sets, such as density functional theory (DFT) methods (B3LYP, B3P86, and B3PW91) and the full-electron second-order Møller−Plesset method (MP2). The basis sets used for optimizations are 6-311++G**, 6-311++G(3df,2p), and aug-cc-pVTZ. Single-point calculations were also performed for the validation of relevant energy quantities at the coupled-cluster single and double excitations with a perturbational estimate of the triple excitation (CCSD(T)) at the same basis set level (6-311++G**) using the MP2/6-311++G** geometry.

A frequency analysis was performed using the same methods to confirm the optimized geometries to be the minima for the stable species on the global potential energy surface. In addition, some relevant energy quantities such as the hydration energies, the proton affinities, and the hydrated proton affinities, etc., are also directly determined for the isolated IM, the hydrated IM, the protonated IM, or the hydrated and protonated IM molecules from the total energies of the relevant systems. The point charge

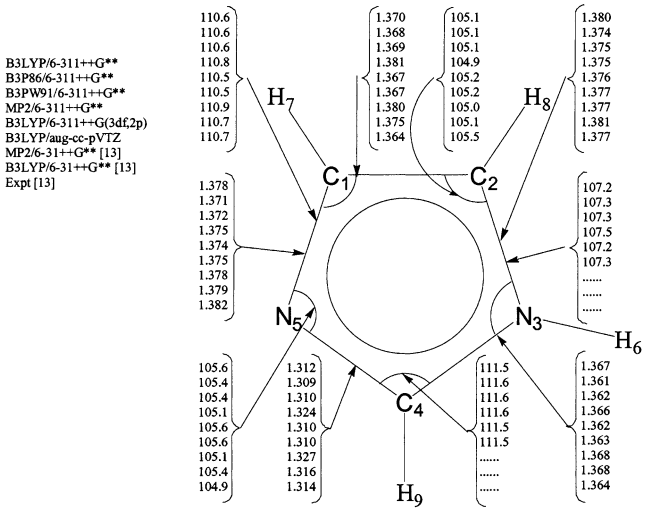


Figure 1. Geometry, optimized and experimental geometrical parameters and the atomic numbering of the free IM molecule.

effect on all energy quantities has been explored using the "CHARGE" model implemented in Gaussian 98.[50,51]

In addition, it should be noted that the effect of the basis set superposition error (BSSE) and zero-point vibrational energy (ZPVE) on various De's was also checked. The results indicate that although these corrections could improve these energy quantities, they could not change the relative regularity among them because the contributions from these corrections are significantly smaller than the corresponding uncorrected values. Therefore, in this work, only the energy quantities regarding the isolated IM without the effects arising from the surrounding are corrected by BSSE and ZPVE for the validation of our preliminary analysis, and no any corrections are considered for all other energy quantities

All calculations were performed with the Gaussian 98 program package[52] on an SGI Origin 3400 parallel supercomputer and Pentium IV/2.4GHz microcomputers.

III. Results and Discussion

1. Inert Nature of H-unit Release from Imidazole. The IM ring in the histidine residue is an important H-unit carrier since it not only can accept a H-unit but can also donate a H-unit in a proton transport chain. Before discussing the energetics of H-unit release from the IM ring in various water-solvated and charge surroundings, it is helpful to investigate the inert nature of the H-unit release process of isolated IM. The geometries, harmonic frequencies, and protonation/deprotonation energies have been determined at several levels of theory with different basis sets for the isolated IM molecule. The main geometrical parameters optimized at the given levels are shown in Figure 1 together with recent experimental data. Comparison of the theoretical results calculated in this work with experimental data and other theoretical values[6−10] indicates that all four levels of theory yield bond distances and bond angles quite close to the gas-phase experimental values. The calculated vibrational frequencies are also in good agreement with the experimental data (see Table 1). Table 2 collects the protonation energies (the proton affinity) and the deprotonation energies (proton De) of IM calculated using different theoretical methods and different basis sets. Lu and Voth[15] calculated the proton affinity to be 238.5 kcal/mol at the MP2/6-311++G(2d,p) level for protonation at the N5-site.[15] At the MP2/6-311+G**//MP2/6-31G* level, Nagy et al.[12] find 231.6 kcal/mol.[12] In addition, at different levels of theory, the calculated proton affinity results were found to be 220 ∼ 228 kcal/mol with vibrational and translation corrections[53] and 216.2 ∼ 222.7 kcal/mol for the proton affinity free energy.[13] These results are close to the corresponding experimental values (223.4 kcal/mol for the former[54] and 214.3 kcal/mol for the latter).[55] The computations show that DFT, wave function correlated MPn (n = 2, 3, 4), and CCSD(T) methods do yield very close results (233.1 ∼ 235.5 kcal/mol), and the deviations among them is within 2.4 kcal/mol except for the MP2 value. The MP2 result is somewhat smaller by 1.6 ∼ 4.0 kcal/mol than the other values. This observation is in agreement with a recent study by El Yazal and Pang[56] in which the MP2 method is found to underestimate the proton dissociation energy of Zinc complexes of imidazole and *N*-methylacetamide, in comparison with coupled cluster methods. Their MP3 and MP4D results are in close agreement with coupled cluster methods, and their DFT calculations also agree well with their coupled cluster results. The data calculated here fall just between Lu's (238.5 kcal/mol) and Nagy's (231.6 kcal/mol) values. Since the DFT calculations are in agreement with the higher-level calculations, and they are much less costly than MP3 and MP4D, they are a method of choice for our studies.

The relative priority for the release of the proton, hydrogen atom, and hydride anion from the N−H site in isolated IM, calculated at B3LYP/6-311++G** level, follows from the values displayed in Scheme 2. The most favorable channel is H-atom release (De 120.96 kcal/mol) and the most difficult is proton release (De 356.26 kcal/mol). This observation is obviously different than what is observed in many biological processes and prompts investigation of factors that contribute to a reversal of this order.

2. Charge Effect on the H-unit Release. Comparison of the three H-unit release processes and the property of the isolated IM implies that the large difference among the De's may, in part, be attributed to whether the electron cloud over the five-membered ring can be effectively distributed to favor the stability of the products after removing the H-unit. Obviously, if a process can decrease the population of the excess electron cloud over the IM ring, it could have a low reaction barrier or small thermal effect. In the three H-unit release processes in Scheme 2, the proton release process leads to greater electron population over the five-membered IM ring. One of the products, H^+, is a strong Lewis acid, and the other is the deprotonated IM ring that is more basic compared with an isolated IM. Therefore, this process should have a large De. For the H-atom release process, the dissociation products are an H atom and an IM free radical. This process reduces the electron population over the five-membered ring, decreasing not only the basicity of the IM ring but also the acidity of the released H-unit. Thus, the release energy should be considerably lower than that in the proton release process. The same is true for the hydride anion release process. Although this hydride release mode further decreases the excess electron cloud population over the IM ring, further weakening the basicity of the IM ring, it significantly increases the basicity of the released H-unit. Therefore, its De should be higher than the H-atom release.

2.1. The Effect of H^+, H, and H^- Bound to the N5-site. The above analysis implies that to promote H-unit release, one could use a Lewis molecule to reduce the basicity or acidity of the products and change the relative priority of the three H-unit releases. In addition to the Lewis molecule catalytic effect, a charge effect may also be recognized from the above analysis. The denser electron population over the IM ring may be bound by the positive charge. To explore the effect from groups with reversed charge on the H-unit releases, a proton, a H-atom, and

TABLE 1: the Vibrational Frequencies Determined Experimentally and Theoretically Using Four Methods (B3LYP, B3P86, B3PW91 and MP2) with Three Different Basis Sets for IM Molecule

This work						Others[a]		Expt	
B3LYP	B3P86	B3PW91	MP2	B3LYP[b]	B3LYP[c]	B3LYP	MP2	Ref [8]	Ref [55]
516	531	530	416	531	540	502	432	551	553
640	645	644	605	646	646	620	576	636	637
677	681	680	660	682	683	656	631	662	664
729	731	731	678	738	741	702	645	735	735
808	810	810	740	825	827	784	710	810	811
865	867	867	785	881	885	841	751	850	852
908	908	906	899	908	907	877	863	892	902
945	946	945	937	947	946	912	901	916	927
1072	1081	1080	1088	1075	1073	1045	1054	1056	1059
1092	1102	1101	1107	1095	1093	1066	1077	1074	1082
1144	1151	1150	1155	1147	1144	1116	1122	1120	1106
1158	1183	1180	1191	1161	1159	1132	1143	1130	1132
1282	1281	1280	1282	1289	1286	1247	1245	1252	1256
1362	1381	1378	1376	1365	1362	1333	1339	1325	1339
1430	1447	1445	1470	1428	1427	1395	1427	1404	1415
1497	1511	1509	1509	1501	1499	1461	1464	1480	1483
1556	1567	1564	1541	1558	1556	1516	1498	1518	1524
3242	3255	3249	3290	3239	3242	3164	3208		3090?
3244	3258	3253	3296	3243	3245	3164	3202		
3273	3287	3282	3318	3271	3273	3192	3226		3114?
3655	3679	3676	3685	3657	3653	3558	3575	3504	3500

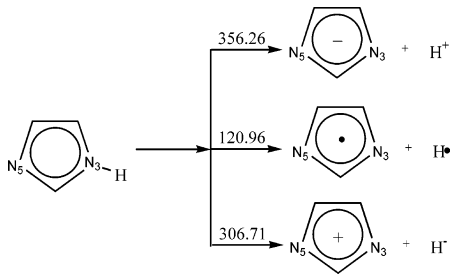
Notes: [a]For other theoretical values, the MP2 values are scaled by a factor (0.96), while the B3LYP values are scaled by the factor (0.97). The used basis set is 6-31++G**. [b] At 6-311++G(3df,2p) basis set level. [c] At aug-cc-pVTZ basis set level.

TABLE 2: the Protonation Affinities (PA), Deprotonation Energies (D_e^P), Dehydrogen Energies (De^H) of the Isolated IM Molecule in Gas Phase Calculated Using Different Basis Sets (in kcal/mol)

	PA(IM)		D_e^P (IM)		D_e^H (IM)	
	uncorr	BSSE−corr	uncorr	BSSE−corr	uncorr	BSSE−corr
B3LYP[a]	233.06	232.79	356.23	356.01	120.96	120.70
B3P86[a]	233.89	233.60	357.44	357.21	124.56	124.34
B3PW91[a]	234.45	234.15	357.96	357.72	120.86	120.63
MP2[a]	231.42		353.14			
MP3[a]	235.75		359.53			
MP4SDQ[a]	235.01		358.45			
CCSD[a]	235.31		358.81			
CCSD(T)[a]	234.05		357.05			
B3LYP[b]	233.58		357.28			
B3LYP[c]	233.85		357.50			
Others			350[d]			
			369[e]			

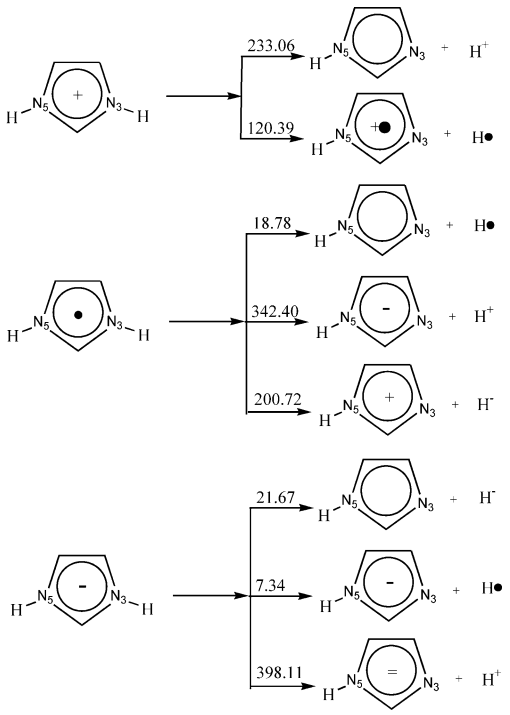
Notes: [a]Using 6-311++G** basis set. [b] Using 6-311++G(3df,2p) basis set. [c] Using aug-cc-pVTZ basis set. [d] Reference 14. [e] Reference 11.

SCHEME 2



a hydride anion are attached to the bare N5-site (cf. Scheme 3). Of course, if a proton couples to the N5-site, the proton De from the N3−H site in the protonated IM is equal to the protonation energy of IM, being 233.06 kcal/mol at B3LYP/6-311++G** level, and should also correspond to the proton De of the N3−H site of the IM ring under the coupling by a proton at the bare N5-site. Comparison of the De between IM and the

SCHEME 3



protonated IM shows the catalytic effect on the De of the N3−H site proton since De decreases by around 123 kcal/mol. However, for the H-atom release mode, the calculated De with and without the proton coupling interaction at the bare N5-site are 120.96 and 120.39 kcal/mol, respectively, indicating that the catalytic interaction by a proton coupling at the N5-site has hardly any effect. Therefore, it can be predicted that a cation coupling at the bare N5-site could significantly improve the proton release from the N3−H site, but would make no contribution to the H-atom release.

To distinguish the electrostatic and the orbital contributions to the improvement of the H-unit De of the N3−H site arising from the coupling at the bare N5-site, an H atom is also

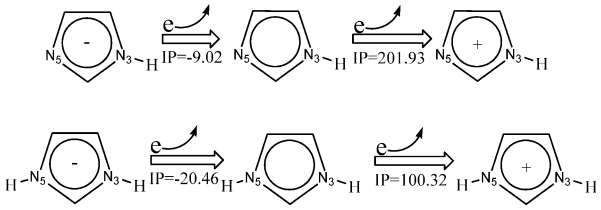


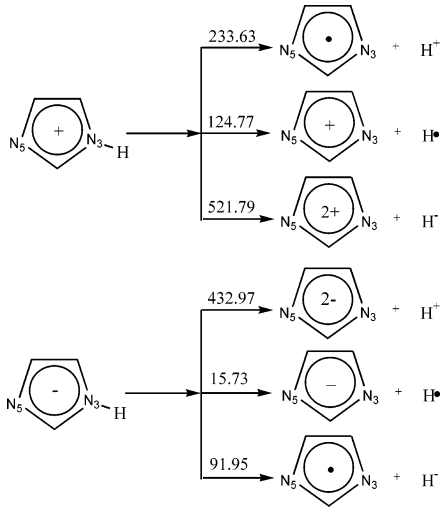
Figure 2. The ionization processes with the corresponding ionization potential (IP).

considered to couple with the bare N5-site (see Scheme 3). The calculated H-unit release energies according to the three release modes are displayed in Scheme 3 as 342.40 (for H^+), 18.78 (for H-atom), and 200.72 (for H^-) kcal/mol, respectively. These results indicate that the H free radical (H-atom) N5-site catalysis greatly decreases the H-atom De, and only slightly decrease the proton De. Similarly, this N5-site coupling interaction has also decreased the H^- De by ca. 100 kcal/mol compared with that in the isolated IM case. Obviously, no explicit electrostatic interaction exists in this scheme, and only the orbital coupling interaction and electron cloud reorganization could influence the H-unit release. Clearly, for IM and the protonated or hydrogenated IM species, a favorable method to promote the H-unit release is to decrease the electrostatic attractive energy between the released H-unit and the remainder obtained by removing the H-unit. The above analysis shows that the factors that catalyze H-unit release are electrostatic interaction, orbital coupling interaction, and electron reorganization effects.

Similarly, a hydride anion is also attached to the bare N5-site (see Scheme 3). Obviously, if a hydride anion could be attached to the bare N5-site, the H-atom and hydride anion releases should be preferred. Inversely, the proton release becomes very difficult. This phenomenon is understandable because the denser electron cloud makes the H-atom and hydride anion releases very easy, and prevents a proton from release. On the other hand, the large negative ionization potential (IP $= -20.46$ kcal/mol) shown in Figure 2 indicates that electron release from the hydride-attached IM is far preferential to an H-atom or a hydride anion release, and it would spontaneously release an electron with the greatest priority.

2.2. The Effect of Charge Injection. In the above cases, a charged particle with different charge amount is used to catalyze the H-unit releases via attaching it to the bare N5-site. To further study the charge effect on the releases, the charge injection method is employed, viz. a charge (one positive charge or a negative charge) is attached to IM, forming a charged IM (see Scheme 4). The injected charge is distributed over the whole IM molecule. Comparison between Scheme 2 and Scheme 4 indicates that positive charge injection significantly decreases the proton De by about 123 kcal/mol. But, it has an inverse effect on the H-atom and hydride anion release. Especially for the H^- release, the De increases by about 220 kcal/mol due to a stronger attraction between the two formed ions. However, for the negative charge injection, the situation is slightly different from that in the positive charge injection. The negative charge injection effect also makes H-atom and H^- release very easy and H^+ release very difficult. Comparison between Scheme 4 and Scheme 3 shows that there is almost the same effect on the De of a proton or an H-atom from the N3−H site with a positive charge injection over the whole IM species as with a proton attached to the bare N5-site. The calculated proton De in the positively charged IM is 233.63 kcal/mol, being almost equal to that (233.06 kcal/mol) in the N5-site protonated IM. The corresponding H-atom De is basically equivalent with only a deviation of ∼4 kcal/mol between two situations. A similar

SCHEME 4



regularity may be observed for the H^- release. Obviously, in these two release modes via proton coupling (with N5-site) or the positive charge injection, both the species that release H-units are monovalent cations. The positive charge effect is predominant in catalyzing the proton release from the N3−H site, but it has little effect on promoting H-atom and H^- releases. However, for the neutral case (the IM vs the N5-site hydrogenated IM), the charge effect is negligible, and orbital coupling and electron cloud redistribution become predominant. The H-atom De goes down significantly by ∼110 kcal/mol after an H-atom is attached to the N5-site of IM compared with that of the IM situation, while the proton De goes down a little (∼14 kcal/mol). But, the corresponding H^- release inversely goes up by ∼100 kcal/mol. In addition, irrespective of whether one negative charge is injected into IM, or an H^- anion is attached to the N5-site, the resulting species are negatively charged. Comparison indicates that in these two different modes to negatively charge the IM, the negative charge effect exhibits a slightly different catalytic role in promoting the H-unit releases. The negative charge injection effect is slightly smaller than the H^- attachment effect. Obviously, the difference between these two charging modes in catalyzing the H-unit releases may be attributed to the fact that the positive charge effect only involves a contribution from the electrostatic interaction, but the negative charge effect involves contributions not only from electrostatic interactions but also from orbital coupling and electron cloud redistribution.

2.3. The Effect of a Point Charge Close to the N5-site. To examine the positive charge effect on the H-unit release from the N3−H site and to distinguish this charge contribution from the overall contribution, a point charge model is used, as implemented in Gaussian 98. Here, the excess proton coupling with the N5-site is replaced by a point charge ranging from 0.0 to 2.0 with an increment of 0.2. This range is motivated by the manner in which the charge localized on the cation attached to the N5-site may vary due to the hydration of and other coupling interactions with the cation. The additional coupling may diffuse a part of the charge carried by the cation to its ligands, and make the total charge localized on the cation decrease.

At the B3LYP/6-311++G** level, the positive charge amount dependence of the N3−H site proton De and the H-atom De have been determined and are displayed in Figure 3. There are two lines for each case, one corresponds to the vertical De and the other to the adiabatic one. For the vertical ones, the five-membered IM rings are kept geometrically unchanged

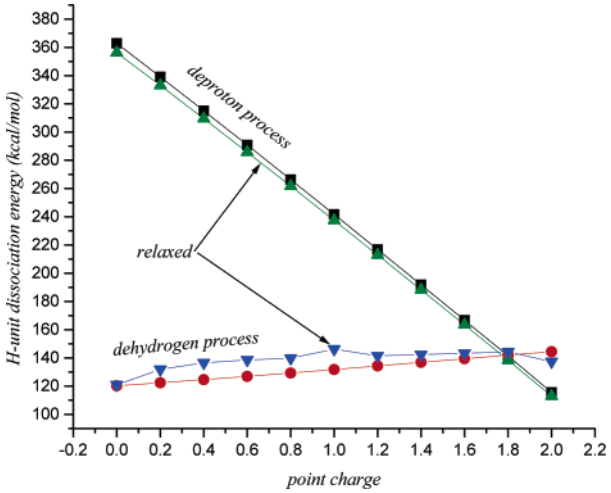


Figure 3. The out-of-N3-site local charge dependence of H-unit De (in kcal/mol).

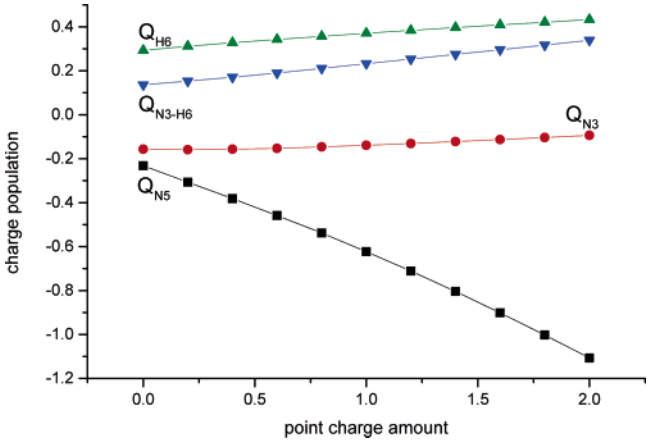


Figure 4. The dependence of charge population over N5, N3, H6 and N3−H6 group on the point charge amount.

before and after removing a proton or an H-atom from the N3−H site, and they are equivalent to the geometry of the optimized protonated IM. The positive point charge lies at the position of the excess proton linked to the N5-site, and the distance between the point charge and the N5 center is fixed at 1.013 Å, the optimized H−N5 distance. For the adiabatic ones, all relevant species are optimized. Namely, on the basis of the optimized IM geometry and the optimized geometries of the deprotonated or dehydrogened IM species, a positive point charge is placed at the point where the distance away from the N5 center is also 1.013 Å for each of them. Comparison of the vertical and adiabatic lines implies that geometrical relaxation effects on De are small, and the deviation between them is ∼6 kcal/mol for proton De. This deviation does not depend on the positive point charge value. But, the deviation between the vertical and adiabatic De's for the H-atom slightly changes subject to the positive point charge amount, being within 0 ∼ 15 kcal/mol. Note that the proton De decreases quickly with an increase of the local point charge while the H-atom De increases slowly. When the positive charge amount increases from 0.0 to 1.0, the proton De decreases by ∼119 kcal/mol, while the H-atom De increases by only ∼25 kcal/mol. When the positive point charge is 1.0, the calculated H-unit De are 237.43 (for proton release) and 146.29 (for H-atom release) kcal/mol, respectively. The former is still significantly larger than the latter, and H-atom release is still dominant. These data are in agreement with the other two methods of positively charging IM, being only slightly larger than the corresponding De (233.06 vs 120.39) of the N5-site protonated system and that (233.63 vs 124.77) of the mono-positive-charge injected system.

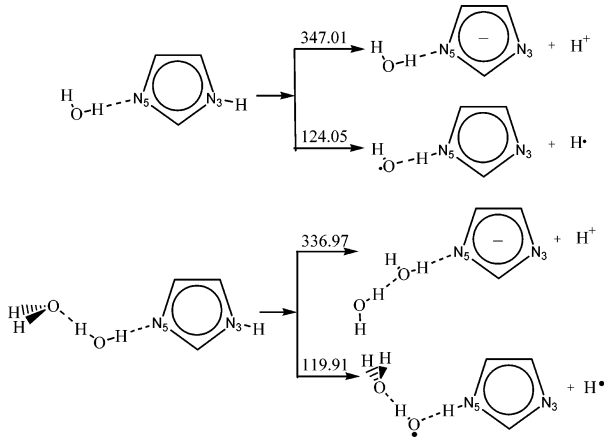
This good agreement among De values determined using three positively charging methods indicates that although the positive charge effect on the proton De is large, just increasing the positive charge up to 1.0 (monovalent cation) cannot change the relative release priority of a proton vs a H-atom without further assistance from other factors. Further increasing the positive point charge amount will cause the two De lines to cross. When the point charge amount reaches ∼+1.8, the two release modes have almost equivalent De (∼140 kcal/mol). This means that in the vicinity of +1.8 of positive charge, the proton release and the H-atom release are essentially competitive if no other catalytic effect is included. After 1.8, the proton release becomes predominant.

The strong catalytic effect of the positive charge on the proton release may be attributed to the strong electrostatic attraction or the neutralization interaction of the electron density over the IM ring, and further to the repulsive interaction to the N3−H site proton. Figure 4 shows the point charge amount dependence of the charge population over the N5, N3, H6 linked to N3 and N3−H groups. The negative charge over the N5-site rapidly increases along with the increase of the positive point charge amount, while that over the N3 center gradually decreases. The positive charge over the H6 to be released gradually increases, so the overall positive charge over the N3−H6 group also gradually increases with the increase of the point charge amount. Inspection of the population over the other C−H groups also indicates a significant inverse proportional dependence of the population of all C−H groups on the point charge amount. These observations imply that increasing the background positive point charge amount may significantly neutralize or reduce electron density and therefore reduce the attractive ability to the released proton from the N−H site and further makes the proton release easier. However, for the H-atom release, because of no explicit electrostatic repulsion interaction between the positive point charge and the released H-atom, the positive charge effect is small. Increasing the positive charge may cause the electron density over the N−H group to decrease, and further increase the binding ability to the electron density. So, proton release followed by an electron cooperative release becomes slightly more difficult.

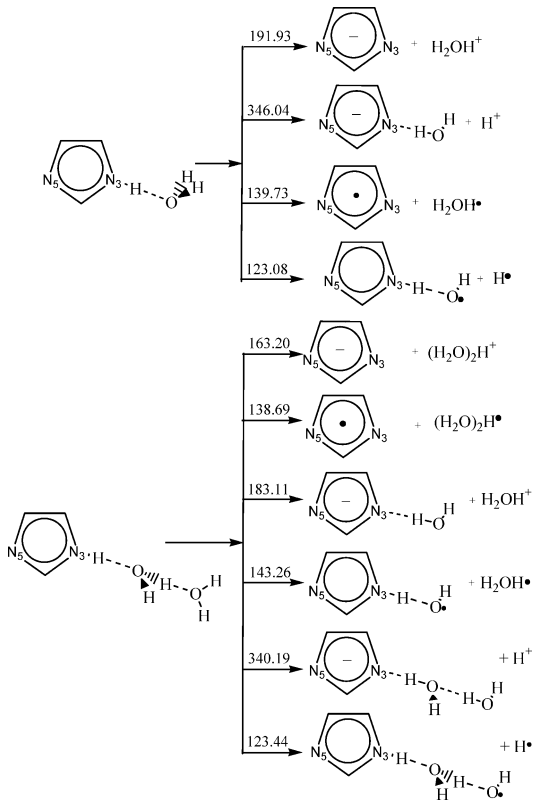
To demonstrate further the dependence of the H-unit De on the point charge position, the H-unit De has also been determined at different distances between the point charge and the N5 center by keeping the point charge amount as 1.0. As anticipated, the proton De exhibits a rapid increase as the point charge-N5 distance increases.

3. Water-catalytic Effect on H-unit Release. The above analysis shows that a positive point charge close to the N5 site decreases the proton De of the N−H site, while not having much effect on H-atom release. Still, the positive charge effect does not change the relative priority of proton and H-atom release until a very large point charge is used. Thus, in this section we focus on the water catalytic effect on the H-unit release of IM by including at one or two water molecules at the N3−H and the N5-sites. The main coupling modes are H-bond interaction. For the N3−H site, the H-bond is the N3−H6···OH_2 mode while, for the N5-site, it is the HO−H···N5 mode. No stable coupling modes with C−H···OH_2 form have been found. The calculated H-bond energies of the monohydrated systems are 6.48 kcal/mol for the former and 7.45 kcal/mol for the latter at B3LYP/6-311++G** level of theory. The N5-site with the bare

SCHEME 5



SCHEME 6

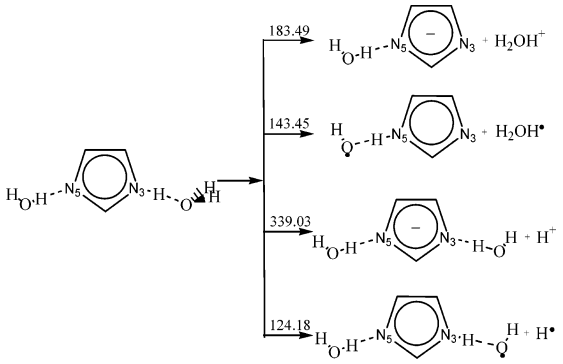


N5: has a slightly stronger ability to be hydrated than the N3-site with N−H form.

To explore the hydration effect on the proton-transfer processes, three kinds of the coupling modes are designed: i) one or two water molecules couple with the N5-site, and the H-unit (H6) releases from the N3−H site (see Scheme 5); ii) one or two water molecules couple to H6, and the H-unit or monohydrated and dihydrated H-units release from the chain linked to the N3−H6 site (see Scheme 6); iii) two waters couple to the N3−H6 site and N5 site, respectively, and the H-unit or monohydrated H-unit releases from N3−H6 site (see Scheme 7).

For case i), Scheme 5 indicates that when a water molecule is H-bonded to the N5 site the calculated H-unit De are 347.01 (H^+) and 124.05 (H atom) kcal/mol. Compared with the unhydrated case, the proton De slightly decreases by ∼9 kcal/mol, but the H-atom De slightly increases by ∼4 kcal/mol. The catalytic effect is far smaller than the charge effect. For the

SCHEME 7



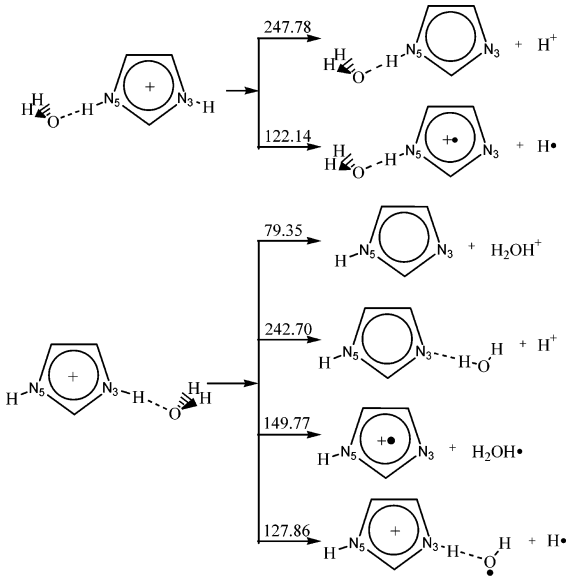
proton release mode, the deprotonated hydrated-IM keeps the same character as that of the product of deprotonation of IM. The only difference is that there is a water molecule bound to the N5-site in the water catalysis mode. For H-atom release, the product is an HO···IM complex instead of a HOH··· dehydrogenated IM. This means that there is an H-atom transfer stage (step) from the bound H_2O to N5-site of the dehydrogenated IM cooperative with the H-atom release from the N3−H site. Spin density analysis also indicates that the unpaired electron spin is located on the HO fragment instead of at the IM moiety. This observation indicates that although the H-atom De of H_2O and IM are almost the same (∼120 kcal/mol), the dehydrogenated IM free radical has a slightly stronger ability to capture a H-free radical than the HO free radical, making the dehydrogenated system stable in this situation. Similarly, using two water molecules to bind to the N5-site, the calculated H-unit De are 336.97 (H^+) and 119.91 (H-atom) kcal/mol. Both of them are smaller than those in the monohydration case, indicating that further hydration can make the two water release processes slightly easier. But the improvement is still small. The dissociation product exhibits the character of a deprotonated IM bound by a two-water-chain at the N5-site for the proton release mode. As occurred in the monohydration case, the product is a complex formed by an IM and hydrated HO free radical for the H-atom release mode. The second water molecule can stabilize the HO free radical group, and further stabilize the product after removing an H-atom from the N3−H site. Therefore, dihydration has an inverse effect (decreasing the De) compared with monohydration, on the H-atom release. The H-atom De in the dihydrated IM is almost equivalent with that of IM.

The results for case ii), (see Scheme 6), are completely different from those in case i. Scheme 6 shows the release modes and the corresponding H-unit De. For the monohydration processes, there are four possible H-unit release modes. The N3−H site H-unit may be released with a bound water, viz. the hydrated H-unit release. The calculated hydrated H-unit De are 191.93 (H_2OH^+) and 139.73 (H_2OH free radical) kcal/mol. These results indicate that single water molecule assisting can significantly decrease the proton De (by ∼164 kcal/mol). The catalytic effect from this mode is far larger than that of the charge effect discussed above. Unfortunately, monohydration increases the H-atom De by ∼19 kcal/mol. Although there is a significant decrease of the proton De, De of a hydrated proton is still larger than that of the hydrated H-atom. In addition to these release modes, the H-units may also be released according to another two modes. Namely, a proton or an H-atom may be released from the terminal water. The dissociation products are the hydrated deprotonated IM and the IM···OH free radical complex, respectively. For the former, when a proton is removed

from the terminal H_2O, the N3−H site proton also cooperatively transfers to the terminal HO group, forming a deprotonated IM···HOH complex. Compared with the proton De of H_2O (395.29 kcal/mol) and IM (356.26 kcal/mol) molecules calculated at the same level, this De (346.04 kcal/mol) of the proton from the terminal H_2O is slightly smaller by only ∼10 kcal/mol than that of IM and significantly smaller by ∼50 kcal/mol than that of a H_2O molecule, indicating this release to be a property of IM instead of that of H_2O. Actually, this H_2O molecule plays just a bridge role to transport a proton for release. However, for the latter, when an H-atom is removed from the terminal H_2O, no H-atom transfer occurs cooperatively from the N3−H site of IM to the O center of the terminal H_2O. The dehydrogenated species may be described as an IM···HO radical complex. The corresponding De is 123.08 kcal/mol. This value reflects the De of a water molecule under a weak perturbation interaction (∼6.5 kcal/mol) from IM compared with the H-atom De of water into OH and H radicals. The property of this H-atom release mode from the terminal H_2O is different from that of the corresponding proton release mode. The former is of the water, and an IM plays a perturbative role, while the latter is of IM, and a H_2O plays a bridging role.

Increasing the water chain length may change the H-unit release property. There are six release modes; the calculated De's are also shown in Scheme 6 (see dihydration case). First, for the N−H bond release mode, the dissociated products are the deprotonated IM and dihydrated proton ($(H_2O)_2H^+$) for the proton release mode and the dehydrogenated IM and dihydrated H-atom ($(H_2O)_2H$) for the H-atom release mode, respectively. The corresponding De for the former is 163.20 kcal/mol, while that for the latter is 138.69 kcal/mol. The water effect from the second water molecule further decreases the N3−H De by ∼28 kcal/mol for the proton mode and hardly changes that for the H-atom mode. This observation may be attributed to the more effective stabilization role of the second H_2O (36.61 kcal/mol of stabilization energy) on the monohydrated proton, H_2OH^+, than that (only 8.93 kcal/mol of stabilization energy) on the monohydrated H-atom, H_2OH. Of course, the effect of the second water also further stabilizes the monohydrated IM species, and the stabilization energy is 7.89 kcal/mol, almost equivalent to that to H_2OH. So the net hydration effect from the second water makes the proton mode De further decrease and the H-atom mode De is unchanged. Similarly, the release may proceed by breaking the intermediate O−H bond. That is, the dihydrated IM may dissociate according to H_2OH^+ or H_2OH modes (see the dihydration case in Scheme 6). The calculated De is 183.11 kcal/mol for the H_2OH^+ mode and 143.26 kcal/mol for the H_2OH radical mode. Although the De (183.11 kcal/mol) of this H_2OH^+ release mode is slightly smaller than that (191.93 kcal/mol) of the monohydrated case, it is greater by ∼20 kcal/mol than that (163.20 kcal/mol) of the dihydrated proton, $(H_2O)_2H^+$. This release pathway is also a property of IM, because there is a cooperative proton transfer from the N3−H site to the middle O center to regenerate a H_2O when a hydrated proton is removed. For the H_2OH radical release mode, the De (143.26 kcal/mol) is also slightly larger than that (138.69 kcal/mol) of the $(H_2O)_2H$ radical mode and that (139.73 kcal/mol) of H_2OH mode in the monohydration case, implying that this mode is slightly more difficult than those. As mentioned above, the N3−H site dihydrated IM may release an H-unit from its terminal H_2O. The corresponding De is 340.19 kcal/mol for the proton release mode and 123.44 kcal/mol for the H-atom release mode. Although the former corresponds to the release property of IM, it is significantly larger than those of the other two hydrated proton release modes ($(H_2O)_2H^+$ and H_2OH^+), indicating that in this dihydrated system, the most difficult release mode for the proton is the proton release from the terminal water, while the favorable release mode is a $(H_2O)_2H^+$ release. Of course, in this terminal H_2O proton release mode, the other two protons also cooperatively transfer from IM to the middle H_2O and from the middle H_2O to the terminal deprotonated H_2O, respectively. The overall consequence is close to the proton release from IM. Inversely, for the H-atom release, the most favorable mode should be that removing an H-atom from the terminal water. Although the dissociated product has an OH radical form weakly bound by a water···IM chain, the H-atom mode is basically equivalent to those in the monohydrated IM and the IM molecules in energy. From the six release modes, it is evident that the hydration effect can significantly increase the stability of the released proton and, as a result, it can significantly decrease the N3−H bond De. But, it inversely decreases the stability of the released H-atom and then increases the N3−H H-atom De for the H-atom release. Summarizing, among the six release modes, the most possible pathway still is the H-atom radical mechanism instead of any of the proton release mechanisms.

SCHEME 8

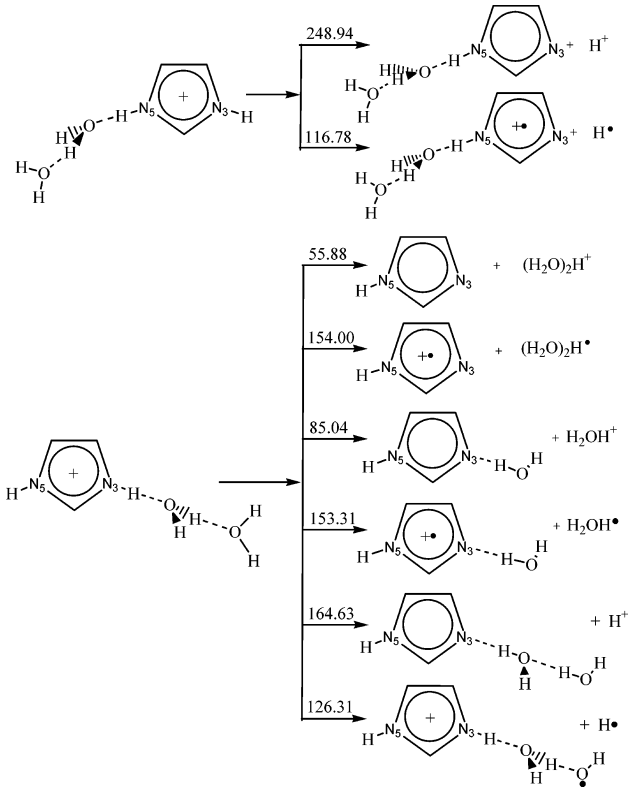


The last dihydration catalysis scheme is case iii), and the results are displayed in Scheme 7. Among the four release modes the most favorable one is to release a H-atom, with a De of 124.18 kcal/mol. The most favorable proton release mode is to release a H_2OH^+, but its De of 183.49 kcal/mol is significantly larger than that of any of H-atom release modes.

4. Water-charge Cooperative Catalytic Effect on the H-unit Release. The above analysis indicates that the charge and water assisting effects can significantly decrease the proton De, but induce little change in the H-atom De. The change from either effect does not change the relative priority of proton versus H-atom release. In this section, we take up the remaining question of how great the combined water-charge catalytic effect is on H-unit release.

4.1. N5-site Protonation and Monohydration. Scheme 8 shows the release modes and the corresponding De's of coupling the water to either the N3−H or the N5−H proton. When a water is coupled to the N3−H proton both the N5−H charge and the water contribute positively to catalyze the N3−H proton release of a hydrated proton (H_2OH^+). The calculated H_2OH^+ release energy is 79.35 kcal/mol, significantly smaller than those

SCHEME 9



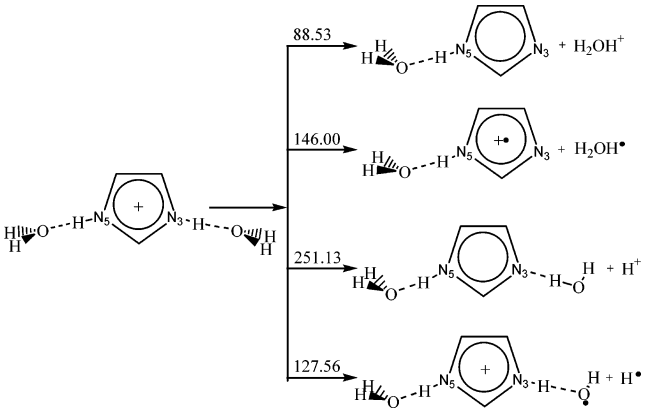
(127.86 vs 149.77 kcal/mol) of the two corresponding H-atom mechanisms. Thus, we have a circumstance where proton release is favored. The most difficult mechanism should be the unhydrated proton release from the terminal H_2O. Of course, its release should be of the N3−H release property instead of the O−H property, because there is a cooperative proton-transfer stage from N−H···O to N···H−O.

On the other hand, if the water is bound to the N5−H site, the situation may be viewed as the coupling of a hydrated proton (H_2OH^+) with the N5-site. As well-known, the acidity of a hydrated proton is significantly smaller than that of a bare proton. Thus, it may be predicted that when a water and a proton cooperatively couple with the N5-site of IM, the catalytic effect should be smaller than that just from the N5-site protonation, and should be larger than that just from the N5-site hydration. The calculated De for this water-proton coupling mode with N5-site is 247.78 kcal/mol for N3−H proton release. This value is greater than that (233.06 kcal/mol) of the N5-site protonated case, and is significantly smaller than that (347.01 kcal/mol) of the N5-site hydrated case. These results confirm the above analysis. The calculated H-atom De is 122.14 kcal/mol, also very close to those of both cases (just hydration and just protonation of the N5-site). Although this hydration-protonation mode has also increased the driving force for the N3−H site proton release compared with the situation of IM, it has not changed the relative priority of proton versus H-atom release. The most favorable release mode is still by H-atom release in the H_2OH^+ catalytic way.

4.2. N5-site Protonation and Dihydration. In view of the significant catalytic effect of water and N5-site protonation on the proton release of the N3−H site, dihydration with N5-site protonation is considered in this Section. The results are summarized in Scheme 9 for the water-dimer coupling case and in Scheme 10 for the separated water coupling case.

If a water dimer is bound to N3−H, the proton De decreases further to 55.88 kcal/mol for the dihydrated proton release form

SCHEME 10



($(H_2O)_2H^+$) relative to the monohydrated proton release. The release as a dihydrated H-atom form is unfavorable, and the corresponding De goes up slightly. Of course, the release as a monohydrated proton (H_2OH^+) form may also be a preferential mode since the corresponding De is 85.04 kcal/mol.

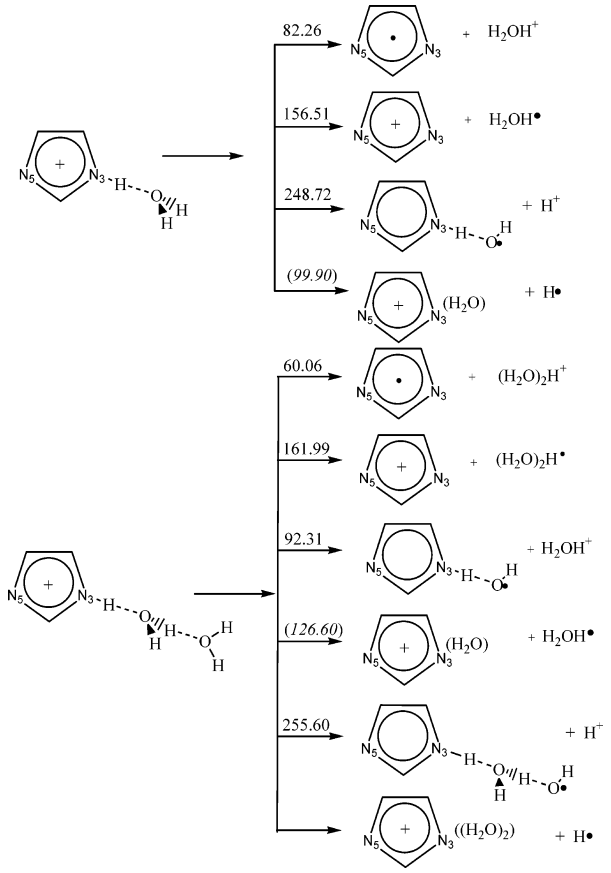
The most difficult release mode is removing a proton from the terminal H_2O, the corresponding De is 264.63 kcal/mol, also being slightly larger than that (242.70 kcal/mol) of the corresponding monohydration assisting mode and that (233.06 kcal/mol) of no water assistance. The most favorable one among the three H-atom release modes should be a H-atom release from the terminal H_2O, and its De is 126.31 kcal/mol. Therefore, the above analysis indicates that, in the water dimer assisting H-unit release from the N3−H site, the most favorable release form produces a dihydrated proton ($(H_2O)_2H^+$).

For a water dimer bound to the H of the N5−H site, the De of the N3−H is 248.94 kcal/mol for proton release and 116.78 kcal/mol for H-atom release. These results are very close to those (247.78 kcal/mol vs 122.14 kcal/mol) in the corresponding monohydration case. The relative priority of H-unit releases is still that the H-atom release is more favorable than the proton release.

Scheme 10 shows the other double-water catalytic modes in which two water molecules are coupled to the two N−H groups. This situation may be viewed as an expanded form of Scheme 8 that uses another water to couple to the remaining N-site. For example, compared with the N3−H monohydration situation, the additional coupling interaction by using a water bound to the N5−H site may distribute a part of positive charge centered at the H of the N5−H group into the additional water, and could slightly decrease the acidity of that H^+. Obviously, this change does not favor the N3−H site proton release, so the calculated De for both H_2OH^+ and H^+ release modes increase slightly, to 88.3 kcal/mol for H_2OH^+ release and 251.13 kcal/mol for the H^+ release. As observed in other situations, the effect from the addition of a second water is basically negligible on the two H-atom release modes (comparing: 127.86 kcal/mol versus 127.56 kcal/mol for the H-atom mode and 149.77 kcal/mol versus 146.00 kcal/mol for the H_2OH mode).

4.3. Charge Injection and Hydration Effect. As discussed above, a positive charge (protonation of the N5 site) and water molecules linked to the N3−H site promote proton release from the N3−H site. Hence, the cooperative effect of both injected charge and water assistance is examined in this section. The release modes are shown in Scheme 11. For the monohydrated case, the easiest release should be of H_2OH^+. Its De is 82.26 kcal/mol and is almost the same (79.35 kcal/mol) as the N5-site protonated case. The easiest H-atom release mode should

SCHEME 11



be free H-atom release from the terminal H_2O, and the corresponding De is estimated to be 99.90 kcal/mol. But, the dissociation product via this mode is a dehydrogenated IM cation with a coupling H_2O molecule at the C4 site (C4···OH_2 coupling), instead of the cation with a H-bond via the N3-site. The other two situations are basically the same as those in the N5-site protonated case, with slightly larger De's. It is evident that proton release as a H_2OH^+ form is more preferential than a H-atom release. For the N3−H site dihydration case, the situation is also similar to that of the N5-site protonation. The corresponding De's in this scheme are slightly larger than those of the N5-site protonation case in Scheme 9. The most favorable release mode also is dihydrated proton release ($(H_2O)_2H^+$) with a De of 60.06 kcal/mol. The next most favorable mode is the monohydrated proton release (H_2OH^+) with a De of 92.31 kcal/mol. These two release modes are also more likely than any H-atom release modes. Therefore, in the N3−H site-coupled water assisting proton release, in addition to N5-site protonation, positive charge injection also improves the proton release. Now the most favorable release form is the hydrated proton.

4.4. Point Charge Model and Hydration Effect. To further explore a positive charge effect on the N3−H site H-unit release, the local point charge model is reexamined with the inclusion of monohydration assistance. Figure 5 shows that the dehydrogenation-hydrated (H_2OH) De increases with a slope ~24 kcal/mol per unit positive point charge while the deprotonation-hydrated (H_2OH^+) De decreases with a slope ~110 kcal/mol per unit positive point charge. Also, the De lines decrease by about 160 kcal/mol relative to the no-hydration case (see Figure 3). Thus, the cooperative effect of the positive local point charge and the water linked to the N3−H proton on the hydrated proton (H_2OH^+) release can significantly improve proton release. The crossing point between the two modes of release has decreased

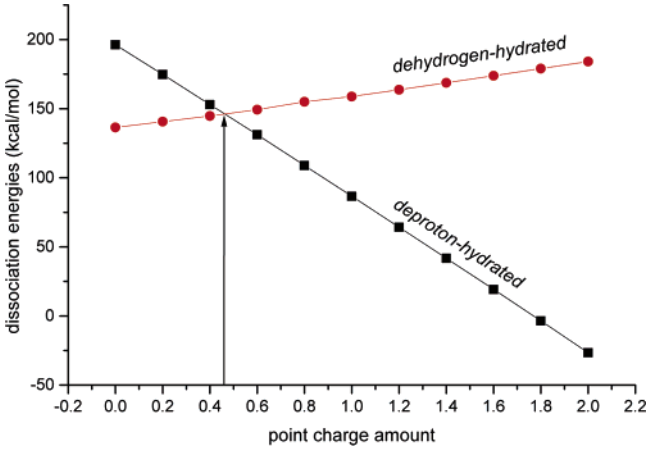
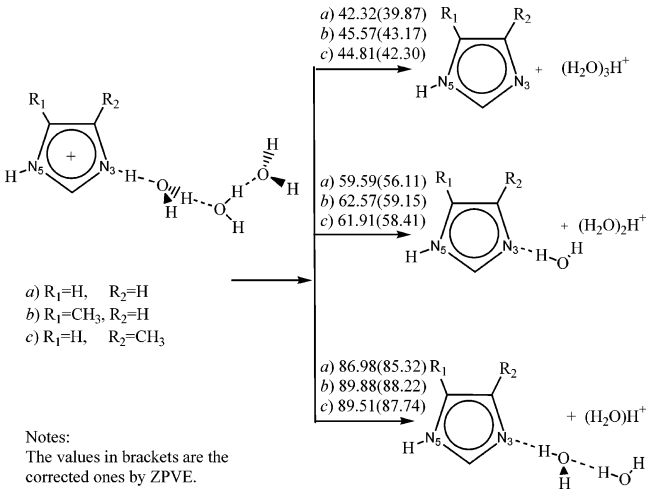


Figure 5. The out-of-N3site local charge dependence of the hydrated H-unit De (in kcal/mol).

to a charge of 0.46. From the monohydration and dihydration effect, it could be predicted that adding a water molecule to the terminal water linked to the N3−H site may further decrease the hydrated proton release energy and move the crossing point to an even smaller charge.

With this result in mind, let us return to the Photosystem II oxygen evolving complex (PSII OEC) given as an example of histidine participation in proton-transfer reactions as both proton acceptor and proton donor.[28−33] In the PSII OEC, upon photoexcitation, an electron transfers from Y_Z, the D1-tyr161, to $P680^+$ and the phenolic proton cooperatively transfers to the bare N5-site of the imidazole side chain of the neighboring D1-his190 residue. The resulting species may be viewed as protonated IM hydrogen bonding back to the tyrosine radical oxygen. This proton-transfer stimulates the N3−H proton release to the other base acceptor (e.g. a carboxylate of a glutamate residue that is in turn hydrogen bonded to other residues and waters).[33] Schematically, this proton-coupled oxidation process of the tyrosine may be viewed as the attack of a proton linked to the donor (the tyrosine radical in above example) to the bare N5-site of IM that causes the release of the N3−H H-unit via the assistance of an acceptor, such as a carboxylate group, water, amino group and so forth. In this case, the attacking H-unit is hydrogen bonded by the conjugate base of the donor, and the conjugate base would be negatively charged if the N3−H H leaves as a proton or a neutral radical if the N3−H H leaves as a hydrogen atom. Because of the coupling interaction from the donor remainder (the tyrosine radical) to the protonated His, the observed positive charge of the proton coupled to the bare N5-site should be smaller than that of a proton coupled to the isolated bare N5-site and, of course, also smaller than the +1 charge of a free proton. Note that the purely electrostatic (versus orbital) effect of the point charge model that we use should be quite descriptive here. That follows because the electron population over the attacking H-unit is between 0 and 1, and there is no additional orbital coupling interaction of the attacking H-unit and the N5-site and the attacking H-unit and the donor to the electrostatic interaction between them. Therefore, the electrostatic effect from the local point charge should be able to account for all coupling interaction contributions to the catalysis for the H-unit release from N3−H. Consequently, Figure 5 can be utilized to predict the nature of the H-unit release, whether it is released as a proton or an H-atom form or, when the product is still a hydrogen-bonded complex, whether the charge distribution over the released H-unit is better characterized as a proton or an H-atom.

SCHEME 12



4.5. N5-site Protonation Trihydration and Methyl Imidazole. The above analyses show that the protonation of or the positive charge attachment to the bare N-site and the water coupling to the N−H site may significantly improve the N−H proton or protonated water release. These models are based only on imidazole and the use of not more than two water molecules. To approach a more protein like environment, we extend the calculations as summarized by Scheme 12. For the case a), three water molecules are involved to assist the proton release. The results indicate that, with proton coupling to the bare N-site, the most favorable channel for N−H proton dissociation is the release of $(H_2O)_3H^+$, instead of $(H_2O)_2H^+$ or H_2OH^+. The De for $(H_2O)_3H^+$ decreases by approximately 13 kcal/mol relative to the analogous dihydrated chain (see Scheme 9). Thus, as one might anticipate, increasing the length of the hydrogen-bonded water chain will decrease the group's dissociation energy. Note also that the De for release of $(H_2O)_2H^+$ in Scheme 12 and the release of $(H_2O)_2H^+$ in Scheme 9 are comparable, emphasizing the role of the stabilization of larger protonated water clusters in decreasing the dissociation energy.

As noted in the Introduction, imidazole can be improved upon as a model of histidine's side-chain. To investigate this issue, a methyl group is used to replace one H linked to a C-site, mimicking both the ϵ and δ forms of the histidine residue, as shown in the b) and c) cases of Scheme 12. The same priority of proton release as in case a) is observed. Importantly, the introduction of a methyl substituent into the imidazole ring does not significantly affect the relevant De. It only increases by 2−3 kcal/mol, and the energy change due to the introduction position is negligible (∼0.6 kcal/mol).

Finally, we have also investigated the effects of including zero-point vibrational energy corrections (ZPVE) and thermal effects to the data displayed in Scheme 12. The values in brackets are just the ZPVE corrections. The ZPVE corrections are basically 2−3 kcal/mol, decreasing the De slightly. The thermal effects decrease the DE by no more than 1 kcal/mol, and are therefore negligible.

IV. Concluding Remarks

On the basis of B3LYP/6-311++G** calculations, the energetics and mechanisms of H-unit release from the N−H site of IM have been explored in detail. IM should be classified as a strong base in the gas phase isolated state. The De's are determined to be 356.26 kcal/mol for proton release, 120.96 kcal/mol for H-atom release and 306.71 kcal/mol for hydride release. Thus, in the isolated state, H-atom release is favored over proton or hydride release. Several mechanisms that favor proton release were considered: namely, protonation of the IM, local and delocalized charge effects, water molecule(s) assisting effects, and combinations of these effects. Our calculations indicate that, individually, protonation of the bare N-site, a positive point-charge localized on the bare N-site and positive charge injection over the whole IM ring can significantly decrease the proton De, but, they do not change the relative priority of the proton release and H-atom release that is found in the un-catalyzed (just IM) situation. Also, these effects on H-atom and hydride anion release are very small; the corresponding De's are hardly changed compared with those in the un-catalyzed situation. Similarly, attachment of a hydrogen atom, or a hydride anion, or negative charge injection over the whole IM ring, significantly decreases both the H-atom and the hydride De, but lead to negligible effects on the proton release. The negative charge injection and hydride anion attachment to the bare N-site are difficult and, even if the injection could be done, electron release from the formed species is the dominant channel.

When a water molecule or two water molecules are coupled with the bare N-site, the improvement is very small on the proton De. However, if a water or a water dimer is bonded to the N−H site, the release of the proton as H_2OH^+ or $(H_2O)_2H^+$ is significantly improved, while the effect on the H-atom release is still small. However, the relative priority of proton and H-atom release is still unchanged. The same is true if two water molecules are bound to the N-site and N−H site, respectively. Thus, protonation of the N5 site or positive charge injection or the water effect assisting effect alone do not change the relative release priority of the proton-unit and the H-atom unit.

To reverse the release priority to favor proton release, a cooperative effect from protonation of the N5 site and water molecule(s) bound to the N_3−H site is required, as shown in Schemes 8, 9, and 12. The presence of one additional water, hydrogen bonded to one water that is hydrogen bonded to the N_3−H, lowers the protonated De by more than 20 kcal/mol and an additional water lowers De further by approximately 13 kcal/mol. The stability of the larger protonated water clusters is mainly responsible for this effect. A stronger base than water would naturally provide an even more dramatic lowering of the protonated channel dissociation energy. Analogous results are found (see Scheme 10 and 11) for the positive charge injection method. The results of replacing an H by a methyl group at −C sites (Scheme 12) show that there is a small effect by this replacement. To the extent that the side-chain of histidine is better modeled with this way, we may conclude that the results of our study would also apply to the histidine residue.

In the point charge scan method the proton De depends strongly on the local positive charge amount; the greater the point charge amount, the smaller the proton De. In contrast, the H-atom De slightly increases with an increase of the point charge amount. A crossover occurs between the proton and H-atom De lines as the point charge amount increases. If there is no water assisting effect, the crossing point occurs at +1.8 (see Figure 3) and with the water assisting effect the crossing point moves to ∼+0.4 (see Figure 5). Therefore, it is very important to use a positively charged group to interact with the bare N5-site for the improvement of the proton unit release. In addition, this finding provides a simple, qualitative criterion for characterizing the property of the released H-unit. In the examples cited in the Introduction, the positive charge of a cation coupled to the bare N-site can be viewed as a fractional positive

charge that may change subject to surrounding factors such as oxidation states and conformational fluctuations. These changes can then determine both the probability of H-unit release and in its form, proton or H-atom or hydride anion.

Our calculations suggest that in biological systems proton transport or release will be dominant when the driving force is a cooperative catalytic effect from a proton, or a distributed positive charge, or a particle of sufficiently large positive charge at the bare, donor N-site aided by a basic species at the acceptor N−H site. The various modes of coupling we have considered span pure electrostatic effects as in the charge scan method to what may be viewed as electrostatic and orbital effects in the charge attachment and charge injection methods. The specifics of H-unit release will depend on these different coupling modes of the donor (a Lewis acid, playing a push role) bound to the bare N-site and that of the acceptor group (a Lewis base, playing a pull role). In general, as the donor to the N5 site becomes more basic, the positive charge over the proton attached to the N5-site might be relatively small and, as a result, the catalytic effect on the N3−H site proton release is smaller. Conversely, as the donor becomes more acidic, it has a weaker ability to bind the proton attacking the N5-site, so the positive charge amount over the proton moving to N5-site might be relatively larger. Consequently, the catalytic effect on the N3−H site proton release is larger, and favors proton over H-atom release. Similarly, the Lewis acidity-basicity of the acceptor may also significantly influence the relative priority of the proton versus H-atom release. The more basic the acceptor, the more preferential the release as a proton form, otherwise the more dominant the release as an H-atom form. This base catalytic effect is greater for the proton release than for the H-atom release. It should be noted that proton translocation in biological processes often is from a donor residue to an acceptor residue via a water chain that can transfer a proton, in contrast with proton dissociation processes. The former mechanism requires information regarding the nature of the acceptor, which is not the content of the present work; but the present work can form the basis for such studies.

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