Theoretical Study of Cooperativity in Biotin

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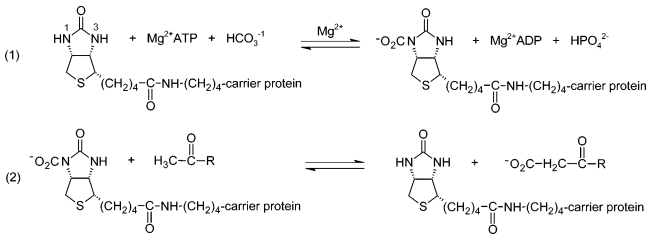
To give a deeper insight into the widely discussed catalytic mechanism of biotin, four representative model molecules and their aggregates hydrogen bonding (H-bonding) to water molecules were investigated by means of ab initio calculations and compared with molecular dynamics simulations. The roles of the ureido group, the sulfur atom, and the side chain of biotin are examined and discussed, respectively. Some significant H-bonding cooperativities are theoretically demonstrated in the ureido group of biotin. The π-electron delocalization of the ureido group makes the system a good candidate for the H-bonding cooperativities, which in turn increases the covalent character of the corresponding H-bonds and facilitates the electrophilic substitution of the nitrogen atoms in the ureido group. The sulfur of biotin may participate in the delocalized π-electron system of the ureido group via special sulfur−nitrogen bonding interactions, which reinforces the H-bonding cooperativities of the ureido group. The side chain of biotin not only reduced the accessibility of 3-NH due to steric hindrance but also enhanced the H-bonding cooperativities of the ureido group by folding over to hydrogen bond to more water molecules. The folded states are a probable way of activating 1-NH by strong cooperative effects. In addition, the H-bonding cooperativities may be a significant reason for the strong and specific binding between biotin and streptavidin.

Introduction

Because biotin serves as a critical enzyme cofactor in a number of important enzymic carboxylations and carboxyl group transfer reactions,[1−3] many experimental works have been carried out to clarify the reaction mechanism. Generally, the following two-step reaction pathway is accepted:



However, the chemical mechanisms of the carboxylations are not well-understood at a molecular level. There still remain several important issues to be examined. For example, the unique structure of biotin has been the subject of speculation with regard to its relationship to mechanism. The imidazolidone ring of biotin has been shown to be appropriate for the reactions in which it is involved. Perrin and Dwyer found that the exchange of the protons attached to the nitrogen atoms of the ureido group occurred sufficiently rapidly for any carboxylation.[4] The bicyclic system has no obvious reason for existence. However, it is unlikely that the structure is not optimal for its purpose.[5,6] In addition, for the carboxylation of biotin to occur, biotin must be deprotonated at the 1-N site.[7] The two NH protons of biotin, the 1-NH and 3-NH protons, are situated at nearly symmetric positions in the ureido ring, but only the 1-NH proton is selectively replaced. Another interesting issue is the reactivity of *N*-carboxybiotin. Biotin preserves a carboxyl group after ATP-dependent carboxylation has occurred, and it readily transfers the carboxyl group to an acceptor. However, biotin is considered to be inherently unreactive, and it must be activated to perform the carboxy transfer reaction.[8]

Detailed knowledge of the conformation, stability, dynamics, bonding nature, electron distribution, and reactivity of biotin will be very valuable in discussing the reaction mechanisms. Yet, only a few theoretical works have been carried out,[9−12] in which some simple compounds were adopted as the models of biotin. In the preceding paper, we carried out molecular dynamics (MD) simulations to study the conformational properties and dynamics of biotin in aqueous solution.[13a] The NMR experiments speculated that there existed some temporary intramolecular H-bonded structures between the carboxyl group of the side chain and the 3-NH proton.[14−16] The simulations confirmed that biotin can form folded conformations with the intramolecular H-bonds via folding over on itself. The purpose of this work was to survey recent quantum chemical calculations and constrained MD simulations upon biotin that attempt to track the gradual differences between isolated hydrogen bonds, hydrogen bonds in clusters of increasing structural complexity, thereby giving a deeper insight into the folding of biotin and the previous issues.

Materials and Methods

The ureido group of biotin is the reaction site, which is important in the CO_2 binding function of biotin. Musashi et al. theoretically investigated various simple model compounds of

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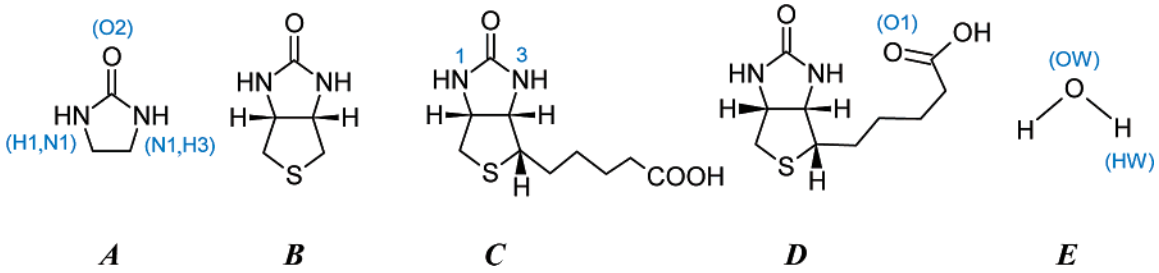

Figure 1. Reference structures of monomers and definitions of atom types.

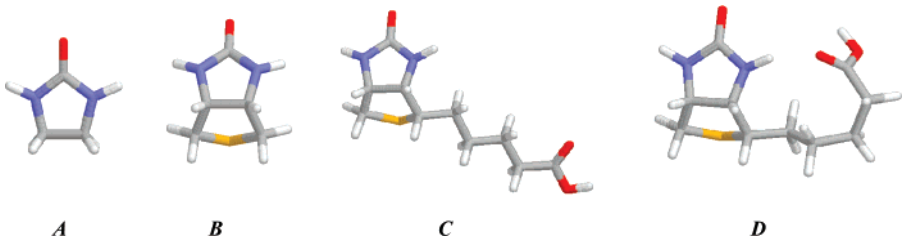

Figure 2. Optimized geometries of monomers. Carbon atoms are gray, nitrogen is blue, oxygen is red, sulfur is yellow, and hydrogen is white.

carboxybiotin and found imidazolidone to be a reasonable model of biotin from the CO_2 binding energy, electron distribution, and frontier orbital energies.[11] In this article, imidazolidone ***A***, tetrahydrothiophene-imidazolidone ***B***, extended biotin ***C***, and folded biotin ***D*** were used as models to analyze their hydrogen bonding (H-bonding) capabilities to water molecules ***E*** (Figure 1), which could give insight into the roles of the ureido group, the sulfur, and the side chain of biotin. The ***C*** and ***D*** conformations were the representative structures taken from the MD simulations.[13a] For computational convenience and expense, the model molecules are assumed to hydrogen bond to not more than two water molecules.

An important concept in the theory of H-bonding is H-bonding cooperativity, which is typically described as the nonadditive enhancement or reduction of one H-bond by the formation of another H-bond with either the proton donor or the proton acceptor of the first H-bond. Considerable attention has been given to the study of cooperative effects.[17−19] The sum of the nonadditive many-body energies has been considered as the energetic contribution of the cooperativity to the stability. There are several ways to measure cooperativity. The energy-based definition of cooperativity is traditionally used to discuss the cooperativity phenomena in intermolecular H-bonding systems. H-bonding energy per H-bond is expressed as

$$\Delta E_n = E(n) - E(n-1)$$

with $E(n)$ and $E(n-1)$ representing the total energies of the aggregates.

Since biotin is selectively carboxylated at the 1-NH proton, only those H-bonds concerning the H1 atom are calculated. A convenient measure of cooperativity is given as[20,21]

$$\Delta\Delta E_n = \Delta E_n - \Delta E_{H1}$$

Here, ΔE_{H1} represents the H-bonding energy of the dimer (such as ***A1*** in Figure 4, ***B1*** in Figure 5, or ***C1*** in Figure 6), in which the H1 atom hydrogen bonds to a water molecule.

To quantify the cooperative effects of the H-bonds concerning the H1 atom, we introduced a coefficient C_{H1} analogue to the ***C1*** coefficient proposed by Koehler et al.[22]

$$C_{H1} = [\Delta E_n - \Delta E_{H1}]/\Delta E_{H1}$$

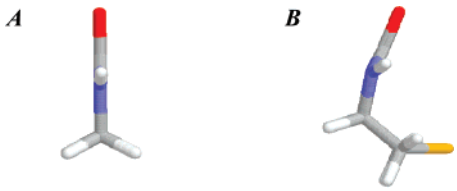

Figure 3. Side elevation of the optimized ***A*** and ***B*** monomers.

A positive value of C_{H1}, which defines the positive cooperativity, means that the H-bonding energy of an aggregate with n H-bonds is greater than that of an aggregate with $(n-1)$ H-bonds. For example, $C_{H1} = 0.10$, namely, a 10% enhancement of the H1···OW H-bonding strength upon formation of another H-bond. Contrarily, a negative value of C_{H1} defines the negative cooperativity.

All the theoretical calculations were carried out using the Gaussian 98 program.[23] The D98** basis set was used to perform HF and DFT calculations. For the DFT calculations, the hybrid B3PW91 method was used, which combines Becke's three-parameter functional with the nonlocal correlation provided by the Perdew−Wang expression.[24,25] The previous methods were adopted to study the H-bonding cooperativities of urea and thiourea, which gave significant results.[26] The same methods were used to compute zero-point energy corrections to the electronic energies. Correction for BSSE was performed using the counterpoise (CP) method of Boys and Bernardi.[27] All of the O2, H1, N1, H3, and N3 atoms in the ureido structure can form H-bonds to water molecules. On the basis of the experimental report,[28] the initial imidazolidone rings of the four model molecules were set to be planar. The initial intermolecular H-bonds between model molecules and water molecules were set to be linear. Then, the geometries were fully optimized.

To analyze the differences of H-bonding between the different conformations of biotin and water molecules, constrained MD simulations of biotin in explicit water were further performed using the TINKER 4.1 molecular modeling package.[29] The three representative initial conformations were extended, semi-folded, and folded biotin, respectively, which were selected from the MD trajectories.[13a] The simulations were performed in the NPT ensemble at $P = 1$ atm and $T = 298$ K with periodic boundary conditions. Each system consisted of one biotin conformation and 498 water molecules. Then, energy minimizations were performed. The systems were optimized with the restraint that

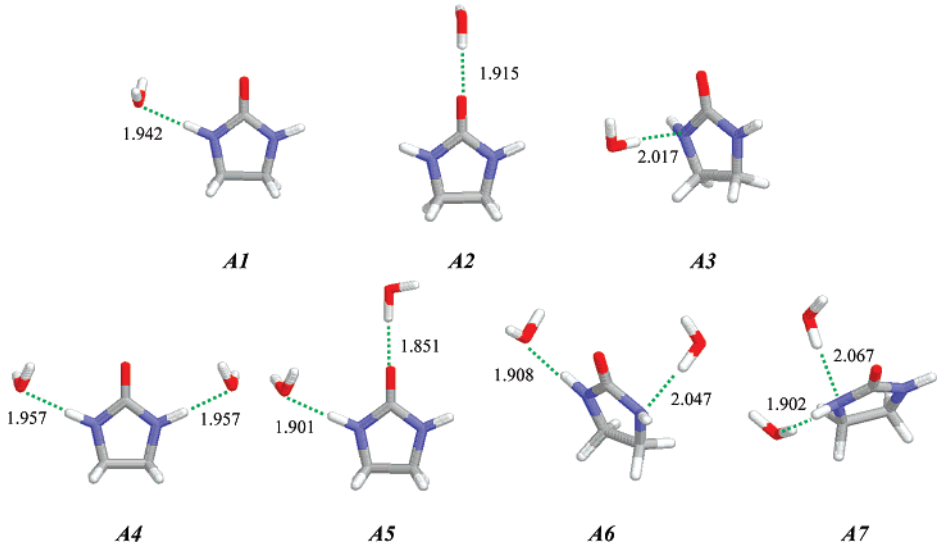

Figure 4. Optimized geometries for the ***A*** aggregates with corresponding H-bond lengths in angstroms.

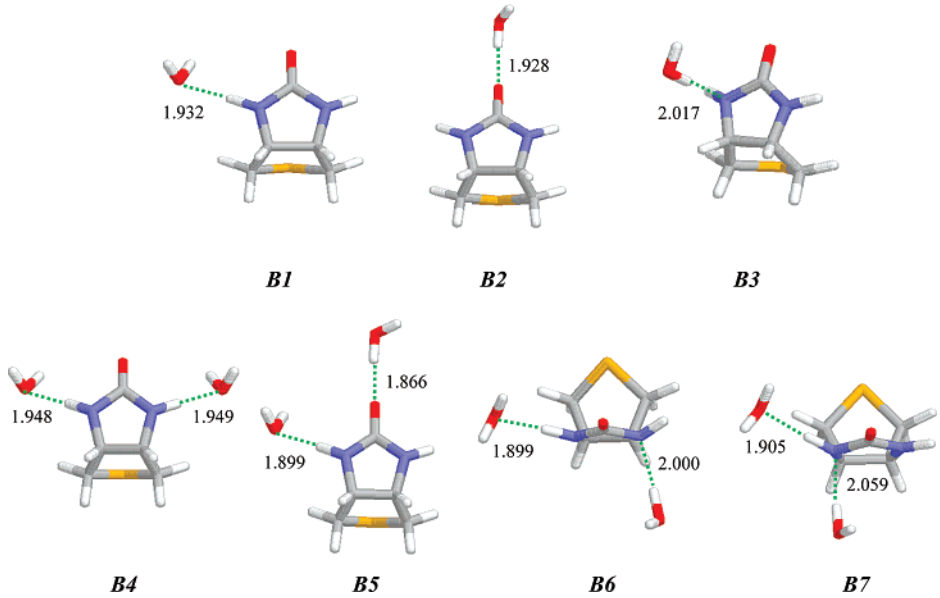

Figure 5. Optimized geometries for the ***B*** aggregates with corresponding H-bond lengths in angstroms.

the geometrical parameters of the initial biotin conformations were kept. The intermolecular geometrical parameters were completely unrestrained. Each system was followed for 2 ns. The first 1 ns was used for equilibration and the rest (1 ns) was used for analysis. Configurations were saved every 0.1 ps. Other detailed descriptions refer to our previous paper.[13a]

Results and Discussion

Monomers. The results for the monomers of the four models and water molecule are summarized in Table 1. Both HF and DFT methods were used to calculate energies and geometries of the model systems. The two methods give similar qualitative trends. In this paper, the HF method was used to illustrate the trends in the electronic charge distribution, and the DFT method was used to interpret other results of energies and geometries. The optimized geometries of the four model molecules are shown in Figure 2. The optimized ***A*** monomer is a symmetric planar structure of a five-membered ring. The optimized ***B*** monomer is still a symmetrical structure; however, the imidazolidone ring and the tetrahydrothiophene ring are not located on a same plane (Figure 3). It seems that the sulfur atom exerts certain long-range interactions to the ureido structure of the imidazolidone ring. Therefore, the planar structures of the imidazolidone ring are slightly destroyed. The optimized ***C*** and ***D*** conformations are very analogous to the MP2 optimized results of Strzelczyk et al.[12] After introducing the side chain, the symmetry of the bicyclic skeleton is entirely broken. There exist some differences between the two NH groups of the imidazolidone ring because of the side chain.

Aggregates of Imidazolidone *A*. The results for the aggregates of imidazolidone ***A*** are collected in Table 2 and Figure 4. The H1(H3), O2, and N1(N3) atoms can hydrogen bond to the oxygen atom (OW) or hydrogen atom (HW) of water, respectively. The atom types refer to those in Figure 1. The binding energies are −3.52 kcal/mol (***A1***), −3.63 kcal/mol (***A2***), and −1.11 (***A3***) kcal/mol, whose corresponding H-bond lengths are 1.942 Å (H1···OW), 1.935 Å (O2···HW), and 2.017 Å (N1···HW), respectively. The O2 and H1 (H3) atoms are found to be better H-bonding participators than the N1 (N3) atom.

The derivatives with modifications in the ureido portion of biotin cannot participate in carboxylase reactions because the ureido portion plays an essential role in the carboxylation

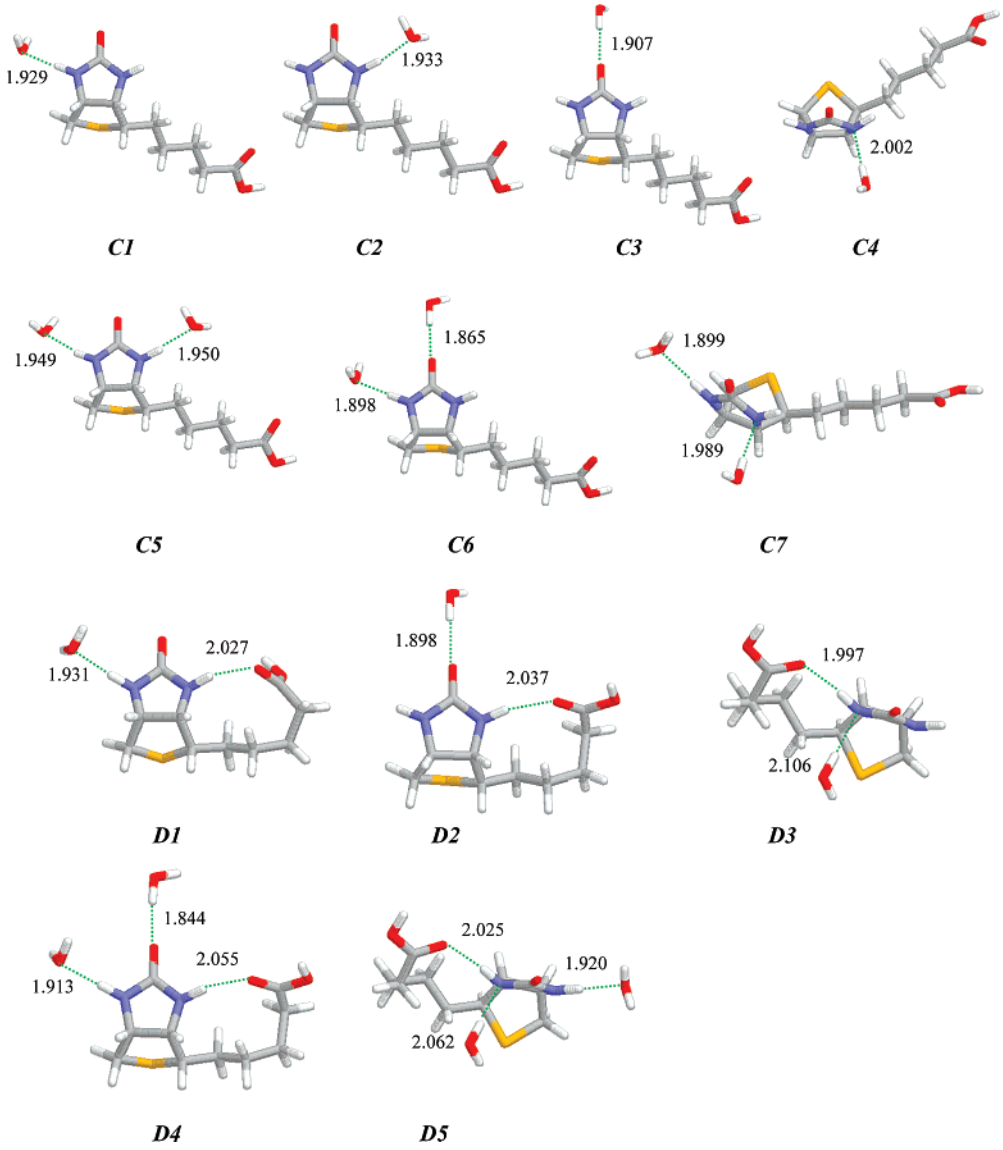

Figure 6. Optimized geometries for the ***C*** and ***D*** aggregates with corresponding H-bond lengths in angstroms.

TABLE 1: Important Energetics, Structural Parameters, and Charge Distributions of Ab Initio Calculations for the Monomers[a]

monomer	method	*E*	H1−N1	H3−N3	q_{H1}	q_{N1}	q_{H3}	q_S
A	HF	−300.840832	0.992	0.992	0.293	−0.457	0.293	
	DFT	−302.523255	1.010	1.010	0.262	−0.294	0.262	
B	HF	−775.228652	0.992	0.992	0.293	−0.457	0.293	−0.034
	DFT	−778.031677	1.010	1.010	0.262	−0.294	0.262	0.037
C	HF	−1118.919331	0.993	0.994	0.292	−0.459	0.289	−0.059
	DFT	−1123.665104	1.009	1.008	0.264	−0.301	0.267	0.003
D	HF	−1118.915762	0.993	0.997	0.284	−0.453	0.336	−0.051
	DFT	−1123.662731	1.009	1.014	0.259	−0.296	0.301	0.006

		E	HW1−OW	HW2−OW	q_{HW1}	q_{HW2}	q_{OW}
water	HF	−76.020875	0.978	0.978	0.330	0.330	−0.660
	DFT	−76.392866	0.963	0.963	0.317	0.317	−0.634

[a] Total energies (*E*) in hartree, other energies in kcal/mol; bond lengths in angstroms; and charge in e.

process. With the ***A1*** dimer as a reference system, H-bonding cooperativities of the ***A4***, ***A5***, ***A6***, and ***A7*** trimers were evaluated and are also shown in Table 2 and Figure 4. The geometrical and energetic parameters were notably altered by the cooperative nature of the H-bond. The behavior of the H-bond length of the trimer is consistent with the energy and electron distribution analysis. In the case of the ***A5*** trimer, the H-bond length *r*(H1···OW) decreases from 1.942 to 1.901 Å, and $C_{H1} = 0.35$, which indicates a strong positive cooperativity and a 35% enhancement of the H1···OW H-bonding strength with respect to the ***A1*** dimer. Thus, the H1 atom of the ***A5*** trimer becomes increasingly polarized. The ***A6*** and ***A7*** trimers also appear to have positive cooperativities, whose C_{H1} values are 0.08 and 0.04, respectively, but are not as strong as that of the ***A5*** trimer. In the case of the ***A4*** trimer, the situation is opposite to the other three trimers. The H-bond length *r*(H1···OW) is enhanced by 0.015

TABLE 2: Important Energetics, Structural Parameters, and Charge Distributions of Ab Initio Calculations for the A Dimers and Trimers

dimer	ΔE	CP	ΔE(CP)	q_{H1}	q_{N1}
A1	−4.59	1.07	−3.52	0.338	−0.463
A2	−4.90	1.27	−3.63	0.302	−0.447
A3	−2.21	1.10	−1.11	0.301	−0.446

trimer	ΔE	CP	ΔE(CP)	ΔE_3(CP)	$\Delta\Delta$E(CP)	C_{H1}	q_{H1}	q_{N1}	H1···OW
A4	−9.02	2.31	−6.71	−3.19	0.34	−0.10	0.332	−0.466	1.957
A5	−10.84	2.45	−8.39	−4.76	−1.24	0.35	0.346	−0.473	1.901
A6	−7.29	2.41	−4.88	−3.77	−0.25	0.07	0.346	−0.458	1.908
A7	−7.18	2.41	−4.77	−3.66	−0.14	0.04	0.345	−0.495	1.902

TABLE 3: Important Energetics, Structural Parameters, and Charge Distributions of Ab Initio Calculations for the B Dimers and Trimers

dimer	ΔE	CP	ΔE(CP)	q_{H1}	q_{N1}	q_S
B1	−5.17	1.25	−3.92	0.329	−0.459	−0.042
B2	−4.25	1.27	−2.98	0.292	−0.456	−0.027
B3	−2.90	1.10	−1.80	0.294	−0.441	−0.029

trimer	ΔE	CP	ΔE(CP)	ΔE_3(CP)	$\Delta\Delta E$(CP)	C_{H1}	q_{H1}	q_{N1}	q_S	H1···OW
B4	−9.89	2.39	−7.50	−3.58	0.34	−0.09	0.323	−0.460	−0.051	1.948
B5	−11.10	2.44	−8.66	−5.68	−1.76	0.43	0.340	−0.460	−0.037	1.899
B6	−8.67	2.30	−6.37	−4.57	−0.65	0.16	0.341	−0.450	−0.038	1.899
B7	−8.37	2.30	−6.07	−4.27	−0.35	0.09	0.337	−0.507	−0.039	1.898

Å and $C_{H1} = -0.09$, which denotes a negative cooperativity and a 9% reduction of the H-bonding strength with respect to the ***A1*** dimer. Even in the case of the negative cooperativity, the formation of a second H-bond is overall energetically favorable as compared to that without the second H-bond. As shown in Table 2, the interaction energy ΔE(CP) of the ***A4*** trimer is −6.71 kcal/mol, that is, 3.19 kcal/mol lower than that of the ***A1*** dimer. These results indicate that the ureido structure of biotin is energetically favorable to form H-bonds to water molecules. The positive cooperative effects result in a greater reduction in the H1···OW distances, which facilitates the ureido group to be deprotonated at the N site.

Aggregates of Tetrahydrothiophene-Imidazolidone *B*. The role of sulfur in biotin has been a long-standing question. The results of the aggregates of tetrahydrothiophene-imidazolidone ***B*** are exhibited in Table 3 and Figure 5. The geometrical and energetic changes in the ***B*** aggregates are more prominent than those in the ***A*** aggregates. The planar imidazolidone ring on biotin is structurally suited for controlling the reactivity of the carboxylations.[30] Examination of the reported structures of biotin and its analogues suggested that the ring with the sulfur was able to precisely adjust the geometry to hold the imidazolidone ring planar. X-ray studies of the analogues in which the sulfur was replaced by oxygen or carbon resulted in structures in which the imidazolidone ring was distorted.[28] *O*-Heterobiotin has generally been found to have no activity or significantly reduce activity in enzymatic carboxylation reaction. Fry et al. reported the unusual kinetics of the exchange of the 1-NH proton of biotin with water protons.[14] Unlike all other amide NH protons, the exchange of the 1-NH proton showed a second-order dependence on $[H^+]$. This unprecedented kinetic behavior required the presence of sulfur. Therefore, Fry et al. speculated that the sulfur could exert its effect by some transannular bonding to the carbonyl carbon of biotin. It seemed that the sulfur in biotin appeared to increase the basicity and nucleophilicity of 1-N, promoting carboxylation at this position. However, these results did not clarify the essence of sulfur in biotin.

Trends in electronic charge distribution may provide some additional insight into the H-bonding structure. In the case of ***B***, the calculated charges of the H1, N1, and S atoms are 0.293, −0.457, and −0.034, respectively. Because of the strong electronegativity of the water oxygen, the charge of the H1 atom increases from 0.293 to 0.329 in the ***B1*** dimer. Especially interestingly, the charge of N1 is −0.459, which hardly changes relative to the ***B*** monomer. Furthermore, the charge of the S atom decreases from −0.034 to −0.042. Analysis of other dimers can also give similar results, which are quite different from those of the ***A*** aggregates. It seems that the sulfur participates in the delocalized electron system of the ureido group, thereby allowing charge transfer from the sulfur atom to the two nitrogen atoms.

This interesting bonding situation makes us relate the celebrated examples of sulfur−nitrogen compounds, $S(NR)_2$ and $S(NR)_3$.[31−33] As imide analogues of SO_2 and SO_3, they did not obey the eight-electron rule. Since they were synthesized, the bond mode of the S−N bonds has been of particular interest. To elucidate the binding nature of the widely discussed hypervalent sulfur−nitrogen species, Leusser et al. recently used high-resolution low-temperature X-ray diffraction experiments and theoretical calculations to investigate four representative compounds.[34] The two model compounds indicated a S^+-N^- bonding mode, while the other two model compounds presented characteristics of a π-system. X-ray studies of biotin have shown that the distances between the sulfur atom and the two nitrogen atoms are only 3.31 and 3.36 Å, respectively.[28] In the case of ***B***, the DFT calculation yields a corresponding distance of 3.38 Å, which is less than the sum of the van der Waals radii. Fry et al. speculated that the sulfur atom could exert its effect by transannular bonding to the carbonyl carbon of biotin.[14] Our calculations reveal substantial π-electron delocalization and charge transfer between the sulfur atom and the two nitrogen atoms. The sulfur may participate in the delocalized π-electron system of the ureido group via the aforementioned particular S−N bonding interactions, which offsets electron delocalization of the nitrogen atoms.

The electron delocalization between the ureido group and the sulfur makes ***B*** a good candidate for the cooperative effect, which provides additional protonation at the nitrogen atoms. With the ***B1*** dimer as a reference system, H-bonding cooperativities of the ***B4***, ***B5***, ***B6***, and ***B7*** trimers were also evaluated

TABLE 4: Important Energetics, Structural Parameters, and Charge Distributions of Ab Initio Calculations for the C and D Dimers and Trimers

dimer	ΔE	CP	ΔE(CP)	q_{H1}	q_{N1}	q_S
C1	−5.07	1.12	−3.95	0.338	−0.458	−0.067
C2	−5.03	1.29	−3.74	0.283	−0.451	−0.082
C3	−4.34	1.24	−3.10	0.296	−0.455	−0.057
C4	−2.02	1.16	−0.86	0.292	−0.441	−0.054
D1	−4.91	1.22	−3.69	0.327	−0.463	−0.057
D2	−6.74	1.31	−5.43	0.289	−0.455	−0.049
D3	−4.84	2.28	−2.56	0.288	−0.452	−0.061

trimer	ΔE	CP	ΔE(CP)	ΔE_3(CP)	$\Delta\Delta E$(CP)	C_{H1}	q_{H1}	q_{N1}	q_S	H1···OW
C5	−9.59	2.80	−6.79	−3.05	0.90	−0.23	0.331	−0.469	−0.075	1.949
C6	−11.04	2.39	−8.65	−5.55	−1.60	0.41	0.345	−0.464	−0.064	1.898
C7	−7.70	2.33	−5.37	−4.51	−0.57	0.14	0.338	−0.450	−0.063	1.899
D4	−12.49	2.49	−10.00	−4.57	−0.88	0.24	0.337	−0.465	−0.055	1.913
D5	−10.04	3.34	−6.70	−4.14	−0.45	0.12	0.333	−0.463	−0.069	1.920

and are shown in Table 3. Their C_{H1} values are −0.09, 0.43, 0.16, and 0.09, respectively. The ***B*** aggregates represent more notable H-bonding cooperativities than the ***A*** aggregates, especially those with N···HW H-bonds. For example, ***B6*** (C_{H1} = 0.16) and ***B7*** (C_{H1} = 0.09) have about twice as many enhancements of H-bonding strengths as the corresponding ***A6*** (C_{H1} = 0.07) and ***A7*** (C_{H1} = 0.04), respectively. The increased H-bonding cooperativities may benefit greatly from particular S−N bonding interactions.

Aggregates of Extended Biotin *C* and Folded Biotin *D*. The results for the aggregates of extended biotin ***C*** and folded biotin ***D*** are displayed in Table 4 and Figure 6. In the case of the ***C1*** dimer, the calculated charges of the H1, N1, and S atoms were 0.338, −0.458, and −0.067, respectively. In the case of the ***C3*** dimer, the corresponding charges were 0.296, −0.455, and −0.057, respectively. In the case of the ***C4*** dimer, the corresponding charges were 0.292, −0.441, and −0.054, respectively. They are very close to those of ***B1***, ***B2***, and ***B3***. Analysis of the corresponding H-bond lengths can also obtain similar results. The side chain of the extend biotin ***C*** does not exert an obvious influence on the H-bonding capabilities of the ureido group but causes a slight difference in the two NH. When the extended ***C*** conformation folds to become the ***D*** conformation via intramolecular H-bonding, the corresponding steric hindrance reduces the accessibility of 3-NH.[13b] The net energetic gain associated with the intramolecular H-bonding formation has to compete with various factors that oppose H-bonding ring closure, such as torsional strain, long-range nonbonded repulsion, entropy, and distortions in covalent bond lengths or angles. Therefore, the strength of the H3···O1 intramolecular H-bond [r(H3···O1) = 2.027 Å] in the ***D1*** dimer is weaker than that of the intermolecular H-bond [r(H3···OW) = 1.950 Å] in the ***C5*** trimer.

With the ***C1*** dimer or the ***D1*** dimer as a reference system, H-bonding cooperativities of the ***C*** trimers and ***D*** trimers are also evaluated in Table 4 and Figure 6. In the case of the ***C5*** trimer, the H-bond length r(H1···OW) increases from 1.929 to 1.949 Å due to H-bonding cooperativity. In contrast, the H-bond length r(H1···OW) of the ***D1*** dimer only increases from 1.929 to 1.931 Å, indicating that the negative cooperativity induced by H3···O1 intramolecular H-bonding is less evident. The C_{H1} values of ***C6*** and ***C7*** are 0.41 and 0.14, respectively, which are close to those of the corresponding ***B5*** and ***B6*** trimers. As expected, the ***D4*** trimers and ***D5*** trimers still produce positive cooperative effects, and the H1···OW H-bonding strengths increase by about 24% (C_{H1} = 0.24) and 12% (C_{H1} = 0.12), respectively.

Analyzing the previous aggregates in detail, we discovered that the formations of the H1···OW H-bonds in turn generate positive cooperativities to the N3···HW, N1···HW, and O2···HW H-bonds. Certainly, they still produce negative cooperativities to the H3···OW H-bond. These reflect the original meaning of the word cooperativity. Now, we can make a conclusion as to the H-bonding cooperativity of the ureido group. There are two donors (H1 and H3) and three acceptors (O2, N1, and N3) in the ureido group. When one donor and one acceptor simultaneously hydrogen bond to other atoms, both the two H-bonds will be strengthened, indicating positive cooperativities. The H-bonding system induced by electron-attracting interactions and electron-donating interactions facilitates the transfer of electron density from one molecule to another, which reinforces covalent characters of the corresponding H-bonds. Hence, both the H-bonds will benefit greatly from the π-electron delocalization of the ureido π-system (−N−CO−N−) representing positive cooperativities. Contrarily, when two donors or two acceptors simultaneously hydrogen bond to other atoms, the H-bonds will be weakened, representing negative cooperativities due to the competition between the two electron-attracting or -donating interactions.

As Compared to Constrained MD Simulations. The MD simulations indicated that biotin in aqueous solution is highly flexible and jumps between extended, semi-folded, and folded states.[13a] The hydrophilic ureido and carboxyl groups of biotin can interact with water to form strong intermolecular hydrogen bonds. Biotin can also form intramolecular hydrogen bonds by folding over on itself. Here, a geometric criterion was adopted to estimate the number of H-bonds. Two molecules are considered to be hydrogen bonded if their separations are such that r(O···H) < 2.45 Å, r(O···O) < 3.60 Å, and the angle H−O···O < 30°.[35,36] A summary of the MD statistics is given in Table 5. As a result of the steric hindrance of the side chain, the semi-folded biotin forms less H-bonds at the O2, N3, and N1 positions than the extended biotin. But once biotin folds over on itself by intramolecular H-bonds, the folded conformation will form more H-bonds than the semi-folded one. Furthermore, the O2, N3, or N1 atoms of the folded biotin favor the formation of two or three H-bonds to water molecules. In the folded state, the percentage of the O2 atom with three H-bonds reaches 1.0%, and the percentage of the N3 and N1 atoms with two H-bonds reaches 2.3 and 4.9%, respectively, which are greater than those in the semi-folded or extended states.

The steric hindrance of the side chain seems responsible for the difference between 1-NH and 3-NH.[13b] However, it is important to point out that the side chain also greatly increases

TABLE 5: Fractions (%) of Biotin Accepted H-Bonds to Water[a]

	O2···HW			N3···HW			N1···HW		
accepted H-bonds	F	SF	E	F	SF	E	F	SF	F
0	18.8%	23.3%	22.7%	37.6%	38.3%	33.5%	26.4%	35.0%	22.0%
1	57.7%	59.6%	57.2%	60.1%	59.7%	64.9%	68.7%	62.0%	73.7%
2	22.5%	16.4%	19.9%	2.3%	2.0%	1.6%	4.9%	3.0%	4.3%
3	1.0%	0.7%	0.2%	0.0	0.0	0.0	0.0	0.0	0.0
av	1.04	0.94	0.97	0.65	0.64	0.68	0.79	0.68	0.83

[a] F, SF, and E denote folded, semi-folded, and extended states of biotin, respectively.

the H-bonding capability of 1-NH on the basis of NMR data.[14−16] Therefore, it is not appropriate to attribute the difference to slowing down the 3-NH exchange rate due to steric hindrance. Instead, it should be attributed to speeding up the 1-NH exchange to a certain effect of the side chain. The MD simulations reveal that when the side chain folds to be close to the ureido ring, the hydrophilic carboxyl and ureido groups gather around to be a greater hydrophilic center and attract more water molecules. The H3···OW H-bond similar to that in the ***C6*** trimer is replaced by the H3···O1 H-bond similar to that in the ***D1*** dimer, which makes the negative cooperativity decrease. More importantly, the O2, N3, and N1 positions attract more water molecules to form abundant H-bonds, which generate additional positive cooperativities. The strong cooperative effects will increase the N1−H1 bond length while causing a greater reduction in the H1···OW distances, which facilitates electrophilic substitution of 1-NH. The H-bonding cooperativity combined with the dynamics behavior of biotin can explain the NMR experiments well.

The chemical mechanism of carboxylation has attracted considerable speculation, particularly regarding how biotin, a poor nucleophile, is induced to react with bicarbonate, a poor electrophile. Perrin and Dwyer speculated that the CO_2 group of carboxybiotin was considered to be activated by a proton donor or cationic species.[4] Thatcher et al. proposed that the carboxyl group became reactive by rotation around the N−CO_2 bond.[9] Sanchez et al. suggested that the noncovalent interaction between the carbonyl oxygen of the ureido group and a Lewis acid could be important in its activation toward carboxylation in the first step of biotin-dependent CO_2 transfers.[37] It is well-known that some modifications in the side chain of biotin render these derivatives inactive as they cannot undergo activation by binding of AMP, a prerequisite for binding to carboxylases.[38−40] We speculate that the folded states of biotin are a probable way of activating 1-NH by the H-bonding cooperativity. When biotin folds via intramolecular H-bonds, the steric hindrance by the side chain reduces the accessibility of 3-NH. At the same time, the folded biotin attracts water molecules to form more H-bonds, whose strong positive cooperative effects will activate the 1-NH protons. Hence, the 1-NH group can be deprotonated more easily by suitable groups of the enzyme in its vicinity. This behavior is exactly what one would expect when there is a switch to control the enzymatic process.

The streptavidin−biotin system is of special interest because it is one of the most tightly bound complexes for noncovalent binding of a protein and small ligand in aqueous solution.[41] Recently, Zhang et al. carried out a full quantum mechanical HF calculation to compute interaction energies for the streptavidin−biotin binding complex.[42] The calculations showed that ab initio binding energies at the HF/3-21G level were almost 30 kcal/mol larger than those given by a force field. The strong H-bonding cooperativities of biotin can reasonably account for the large difference in binding energy. There is a need to develop new molecular force fields that can provide a quantitatively accurate description of H-bonding cooperativity.

Conclusion

Biotin provides a classical case in the context of investigation of enzyme catalysis due to its structural dimension and complexity. In the present paper, imidazolidone ***A***, tetrahydrothiophene-imidazolidone ***B***, extended biotin ***C***, and folded biotin ***D*** were theoretically investigated as models of biotin and compared with MD simulations. A summary of the findings on the structure−function relationships of biotin is as follows.

(1) The imidazolidone ring of biotin is the reaction site of the catalytic process. Our theoretical calculations confirm the existence of significant H-bonding cooperativities in the ureido group of the imidazolidone ring. The strong cooperative effects benefit from the delocalized π-electron system of the ureido group, which promotes the electrophilic substitution of the nitrogen atoms.

(2) The sulfur of biotin appears to increase the basicity and nucleophilicity of the nitrogen atoms, facilitating carboxylation at this position. The sulfur may participate in the delocalized π-electron system of the ureido group via special sulfur−nitrogen bonding interactions, which further reinforces the H-bonding cooperativities of the ureido group and allows additional protonation at the nitrogen positions.

(3) Biotin in aqueous solution is highly flexible and jumps between extended, semi-folded, and folded states owing to the side chain. Although the folded states occur at a few fractions during the simulation, they can be important to the biological significance of biotin. While the carboxyl group of the side chain approaches the ureido group via folding over on itself, the steric hindrance reduces the accessibility of 3-NH. The folded biotin simultaneously attracts water molecules to form more H-bonds, which leads to the crucial H-bonding cooperative effects. The strong positive cooperativities activate the 1-NH protons so that the 1-NH group can be deprotonated more easily. The folding of biotin is likely to act as a switch to control the enzymatic process.

While this work was in progress, Houk and DeChancie investigated the unusually strong reversible binding of biotin−streptavidin using density functional and MP2 ab initio quantum mechanical methods.[43] They found that the origin of biotin−streptavidin hydrogen bond cooperativity was the polarized electronic structure of the ureido moiety of biotin, which caused strong interactions with the first and second contact-shell hydrogen bonding residues. Some results in this paper agree with their investigations.

This article provides more insight into the structure−function relationships of biotin. The $C_{10}H_{16}O_3N_2S$ molecule of biotin possesses a unique bicyclic heterocyclic skeleton to which is appended a functionalized side chain. The biologically active enantiomer further possesses an array of three contiguous stereocenters in the specific all-cis configuration, which enables

the ureido group, sulfur atom, and carboxyl group of the side chain to interact with each other to generate a large delocalized electron system. Slight modifications in the imidazolidone ring, the tetrahydrothiophene ring, or the side chain will cause biotin to be inactive. In contrast, free and bioactive biotin can be released from amino acid or peptide conjugates of biotin with an intact side chain and bicyclic heterocyclic ring. The 32 atoms of biotin perfectly constitute its spatial configuration with minimal atoms and maximal structural complexity.

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