Zinc's Affect on Proton Transfer between Imidazole and Acetate Predicted by ab Initio Calculations

Jamal El Yazal,[†] Rachel R. Roe,[†,‡] and Yuan-Ping Pang*

Mayo Clinic Cancer Center, Tumor Biology Program, Department of Molecular Pharmacology and Experimental Therapeutics, Molecular Neuroscience Program, and Mayo Foundation for Medical Education and Research, 200 First Street SW, Rochester, Minnesota 55905

Received: December 6, 1999; In Final Form: March 17, 2000

We have recently reported proton dissociation energies of common zinc coordinates, in the presence and absence of the zinc divalent cation, that were obtained from large basis set density functional theory calculations, which suggest a possibility of imidazolate as a zinc coordinate in proteins. To investigate this possibility in further detail, we have searched for potential proton acceptors for the zinc-coordinated imidazole group, as potential proton donors in proteins, through a survey of zinc protein crystal structures and ab initio calculations. Herein, we report the result of our survey of the Protein Data Bank and the ab initio calculations using the B3LYP/6-311+G(d,p) and MP2/6-311+G(d,p) methods. The results reveal that the imidazole−acetate dyad is energetically less stable than the imidazolate−acetic acid dyad when the imidazole and imidazolate are coordinating to the zinc divalent cation, and vice versa in the absence of zinc. The results suggest that the carboxylate group of Asp(Glu) at the second coordination shell of a zinc complex in proteins is not a hydrogen bond acceptor but rather a proton acceptor for the zinc-coordinated imidazole.

Introduction

Experimental and theoretical studies of zinc-containing proteins have advanced our understanding of the physical chemistry of zinc coordination in proteins.[1−31] However, the exact details of zinc coordination in proteins are still not well-understood.[15] We have recently reported proton dissociation energies of common zinc coordinates in the presence and absence of the zinc divalent cation obtained from large basis set density functional theory (DFT) calculations and suggested a possibility of imidazolate as a zinc coordinate in proteins.[31] To investigate this possibility in further detail, we have searched for potential proton acceptors for the zinc-coordinated imidazole group as a potential proton donor in proteins through a survey of zinc protein crystal structures in the 1998 release of the Protein Data Bank (PDB).[32,25] We found that 88% of the protein crystal structures containing the zinc-coordinated imidazole have the carboxylate group of Asp and Glu and the phosphate group of nucleic acids that surround the zinc-coordinated imidazole in the vicinity up to 8 Å from the imidazole group in proteins. This finding prompted us to investigate whether the Asp(Glu) residue serves as a proton acceptor or as a hydrogen bond acceptor for the zinc-coordinated imidazole in proteins. Herein, we report the result of our survey of the PDB for the search of proton acceptors for the zinc-coordinated imidazole group in proteins and ab initio calculations, suggesting that the carboxylate group of Asp and Glu at the second coordination shell of a zinc complex in proteins is not a hydrogen bond acceptor[4] but, rather, a proton acceptor for the zinc-coordinated imidazole.

* To whom correspondence should be addressed. E-mail: pang@mayo.edu.
[†] Department of Molecular Pharmacology and Experimental Therapeutics.
[‡] Present address: Maryville College, PO Box 2173, Maryville, TN 37804.

Methods

The ab initio calculations were carried out by using the Gaussian 98 program[33] running on an SGI Origin 2000 (8 × 195 MHz, 2.0 GB memory, and 40 GB disk) and four Origin 200s (8 × 180 MHz, 1.2 GB memory, and 116 GB disk). Geometry optimizations in the singlet and triplet states and harmonic frequencies used to preclude the low-energy transition-state geometries were computed by using the second-order Møller−Plesset (MP2) theory[34−39] or the Becke's three-parameter formulation (B3LYP) density functional theory[40,41] with the 6-311+G(d,p), 6-311+G(2d,2p), or LANL2DZ basis set, briefly described below.[42,43] The quadratic convergence method (SCF = QC)[44] was used to solve numerous convergence problems encountered in energy minimizations. The structures of the imidazole−acetate(formate) dyads and imidazolate−acetic(formic) acid dyads were initially generated with the Quanta program.[45]

The MP2 theory accounts for electron correlation, which is crucial to the present study and can successfully model a wide variety of systems yielding accurate geometries, although it requires excessive computing time and disk space. The density functional theory computes electron correlation through functions of the electron density, which itself is a function of coordinates in real space. This method (e.g., B3LYP) has been shown to describe hydrogen bond interactions reasonably well[46] at only a modest cost of computing time and disk space but far less than the needs by the MP2 method. The LANL2DZ basis set is developed to handle heavy atoms after the third row of the periodic table and treat the electrons near the nucleus in an approximate way via effective core potentials.[43] The 6-311+G(d,p) basis set adds the d functions as polarization functions to heavy atoms (labeled with "d") and the p functions as polarization functions to hydrogen atoms (marked with "p") in order to allow orbitals to change size and shape. The p functions for the hydrogen atoms are important for calculations for cases in

10.1021/jp994283x CCC: $19.00
Published on Web 06/24/2000

which the hydrogen atoms are the sites of interest, as in our study of proton transfer. It also adds the large-size versions of the s- and p-type functions as diffuse functions (noted with "+") to heavy atoms to allow orbitals to occupy a larger region of space. The diffuse functions are necessary for molecules in our study with electrons relatively far from the nucleus, molecules with lone electron pairs and molecules with significant negative charges. Furthermore, it adds an extra three sizes of s and p functions to heavy atoms to model heavy atoms such as zinc. The 6-311+G(2d,2p) basis set is the same as the 6-311+G-(d,p) basis set, with the exception that it adds 2d functions to the heavy atoms and 2p functions to the hydrogen atoms to count the polarization effect.

Results and Discussion

Survey of the PDB. Using the PDB 3DB Browser provided by the PDB World Wide Web site at the Brookhaven National Lab of the USA in 1998, we found 407 crystal structures of zinc proteins documented in the PDB on June 5, 1998. These proteins contain single or multiple zinc binding sites that play either a functional or a structural role. We did not segregate the proteins with catalytic zinc binding sites from the ones with structural zinc binding sites because our objective was to search for proton acceptors for the zinc-coordinated imidazole group as a potential proton donor in proteins. Instead, we selected structures with resolutions higher than or equal to 2.25 Å, at which the side chain structures of the proteins are defined by the electron density map. To avoid bias due to certain proteins such as insulin, which had been extensively studied and had 23 structures documented in the PDB, we used the structure that had the highest resolution and discarded the others of the same protein. We did not use structures that had an irregular zinc complex or in which the zinc ions were used to improve the quality of the crystals (e.g., verotoxin, PDB code: 1BOY). These considerations led us to select 62 structures of zinc-containing proteins (Table 1). Through visual inspection of the 62 structures, we found 51 structures that contained at least one His residue as a zinc ligand, and of those 51 structures, 88% had the carboxylate group of Asp and Glu and other anionic groups that were surrounding the zinc-coordinated imidazole in the vicinity up to 8 Å from the imidazole group in proteins (Table 1). The results suggest that the Asp or Glu residue may serve as a proton acceptor for the zinc-coordinated imidazole, which serves as a potential proton donor in proteins.

Acetate as a Remote Proton Acceptor. To investigate if Asp and Glu can serve as proton acceptors when they are not close enough to form a hydrogen bond with imidazole or to coordinate with zinc in proteins, we calculated the energies of the model systems in which an acetate was 5 to 8 Å away from an imidazole group that was either coordinating or not coordinating to the hydroxide-bound zinc divalent cation (**1**−**6**, see Figure 1) and the energies of the same systems, with the exception that the imidazole was deprotonated and the acetate was protonated (**1′**−**6′**, see Figure 1). In such systems, the structures of imidazole and imidazolate, that each was coordinating or not coordinating to the hydroxide-bound zinc divalent cation, were energy minimized by the B3LYP/6-311+G(2d,-2p) method in the absence of acetate and acetic acid, respectively; likewise, the structures of acetate and acetic acid were individually optimized with the same method in the absence of zinc and hydroxide. The orientations of the acetic acid or acetate relative to the imidazolate or imidazole and the geometries of all these molecules are defined by the values in Tables 2 and 3. The 5 and 8 Å separations between the two molecules were determined on the basis of the above-mentioned survey of zinc protein crystal structures. Energy minimizations of the two molecules together were not pursued for the following reasons. First, at the 5 and 8 Å separations, the structures of the two molecules are more affected by their neighboring residues in proteins than by one another. Second, the ability of acetate to accept the proton from imidazole does not, in principle, depend on the orientations and positions of the acetate relative to the imidazole.

TABLE 1: Search of Proton Acceptors for the Zinc-Coordinated Imidazole as a Potential Proton Donor in Zinc-Containing Protein Crystal Structures

PDB code[a]	proton acceptor	N−O distance[b] (Å)	PDB code	proton acceptor	N−O distance (Å)
1IRN	[c]		1SLM	asp	2.9
1PPT			1AZV	asp	2.8
1XSO	asp	2.8	1CLC	glu	2.6
1EZM	asp	2.7	2NLL		
1AH7	asp	4.7	4ENL		
2CTB	asp	2.7	1FUA	glu	6.0
2CBA	glu	2.6	1PTQ	asp	6.2
1HFC	glu	5.7	1ZIO		
1AAY	PO_4	2.8	1F3Z	asp	6.5
1BTK		> 8.0	1ALK	asp	7.1
2MYR	asp	5.6	1STE	asp	3.4
1KUH	glu	5.4	1BRH	asp	6.6
1ZIN			1TAF		> 8.0
1LAM			1FRP		
8TLN	asp	2.7	1XER	glu	5.2
1KAP	glu	5.6	2EBN		> 8.0
3BTO	asp	2.7	4MT2		
1VHH	glu	2.8	1IAG	glu	5.6
1PMI	asp	5.2	1RMD	glu	3.7
1HML			1CFV		> 8.0
1SAT	glu	5.4	1AUI	asp	3.8
1XJO	asp	3.0	1DPM	asp	2.6
1ENR		> 8.0	1CTT	glu	6.8
1TON	asp	2.7	1LBA		> 8.0
2TCI	glu	5.6	1FRO	gsb[d]	6.5
8RNT			1TSR	glu	3.8
1ATL	glu	5.4	1FT1	asp	2.7
1AST	asp	5.3	1JAQ	asp	4.5
1JAP	glu	5.4	1HXQ	asp	4.3
1PUD	asp	5.9	1LML	glu	5.5
1BME	asp	2.7	1MMQ	asp	4.9

[a] References for protein structures are available in the PDB coordinate files. [b] The N−O distance was defined between the imidazolyl nitrogen atom that was not coordinating to the zinc ion and the carboxy oxygen atom that was closest to the nitrogen atom. [c] The protein structure does not contain the His residue as a zinc ligand.[d] gsb: *S*-benzyl-glutathione.

The single-point energies of **1**−**6** and **1′**−**6′**, calculated with the B3LYP and MP2 theories using the 6-311+G(d,p) basis set, are listed in Table 4. In the absence of zinc, the energies of the systems with an imidazolate are at least 0.9 kcal/mol higher than those of the systems with an imidazole. These results suggest that the carboxylate group of Asp and Glu is not an effective proton acceptor for the imidazole group of His. These results also reveal that the basicity of the carboxylate group *relative to* that of the imidazole does not rely on the relative orientations and positions of the carboxylate group.

In contrast, in the presence of the hydroxide-bound zinc divalent cation, the systems with an imidazolate are at least 53 kcal/mol more stable than those with an imidazole, suggesting that the carboxylate group is an effective proton acceptor for the OH^--Zn^{2+}-imidazole, whose proton dissociation energy is greatly reduced by the zinc divalent cation.[31] On the other hand, consistent with the results of the systems devoid of zinc, the *relative* stabilities of the zinc-containing systems are not

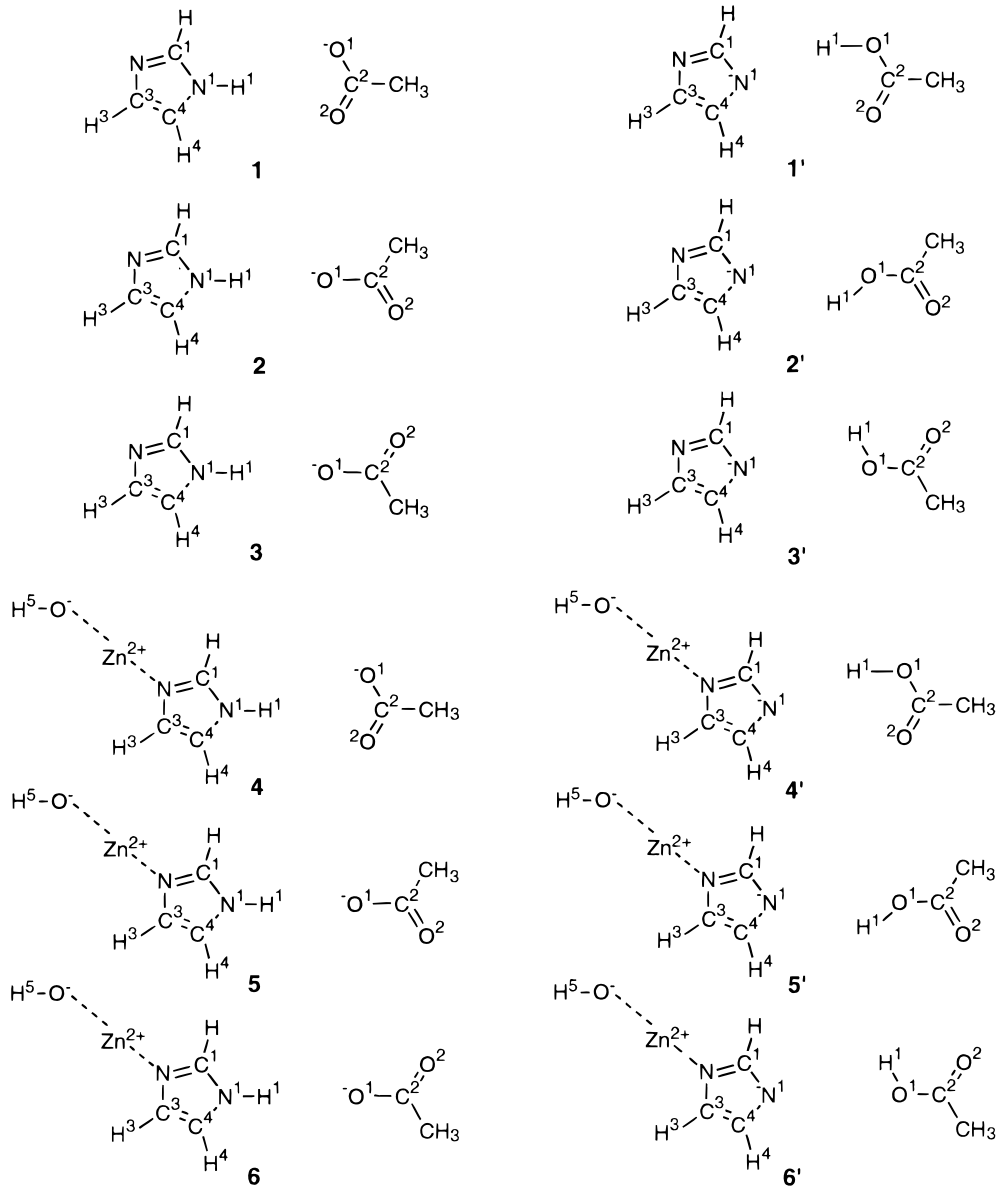

Figure 1. Different imidazole−acetate and imidazolate−acetic acid dyads in the presence and absence of the hydroxide-bound zinc divalent cation (for bond lengths, angles, torsions, and interatomic distances, see Tables 1 and 2).

altered by changes of the relative orientations and positions of the carboxylate group.

Acetate as a Nearby Proton Acceptor. To investigate if Asp and Glu can serve as proton acceptors when they are close enough to form a hydrogen bond with the imidazole but not close enough to coordinate to the zinc divalent cation in proteins, namely, when they are at the second coordination shell of a zinc complex in proteins,[4] we performed calculations of the model systems with the N^1-O^1 distance kept less than 5 Å, so that these molecules were capable of forming an intermolecular hydrogen bond in the three possible ways depicted in Figure 1. To consider the effect of the intermolecular hydrogen bond in each dyad, we optimized the entire dyad with quantum mechanical calculations, although such minimizations involving proton transfer were extremely time-consuming. In the absence of the zinc divalent cation, we found that the imidazolate−acetic acid dyad always converged to the imidazole−acetate dyad after an energy minimization, using either the B3LYP/6-311+G(d,p) or the MP2/6-311+G(d,p) method (Figure 2). In the imidazole−acetate dyad structures that were optimized using the two methods, the N−H bond length of the imidazole is 1.1 Å; the intermolecular hydrogen bond length of the dyad is 1.6 Å; and the hydrogen bond angle of NHO is 178° of arc. The coordinates of the optimized imidazole−acetate dyad structures are provided in the Supporting Information. This result is consistent with the report that a full BLYP/6-31G* optimization starting from a geometry corresponding to a formic acid−imidazolate dyad resulted in a geometry corresponding to a formate−imidazole dyad[47] and the report that the formate-imidazole dyad is more energetically stable than the formic acid−imidazolate, according to the experimental studies.[48] The results suggest that acetate cannot serve as a proton acceptor but rather as a hydrogen bond acceptor for the imidazole that does not coordinate to zinc in proteins.

In contrast, in the presence of a hydroxide-coordinated zinc divalent cation, we found that the imidazole−acetate dyad always converged to the imidazolate−acetic acid dyad after an energy minimization using either the B3LYP/6-311+G(d,p) or the MP2/6-311+G(d,p) method. The use of a hydroxide-coordinated zinc divalent cation rather than a zinc divalent cation was to avoid the move of the acetate toward the zinc divalent cation to form an acetate- and imidazole-coordinated zinc

TABLE 2: Distances (Å), Angles (deg of arc), and Torsions (deg of arc) that Define the Orientation of the Acetate (Acetic Acid) Relative to Imidazole (Imidazolate) in the Dyads Shown in Figure 1

struct	N^1-O^1	$C^1-N^1-C^2$	$N^1-O^1-C^2$	$N^1-C^2-CH_3$	$C^1-N^1-C^2-O$
			separation at 5 Å		
1	4.993	128.64	102.97	179.83	−179.8
1′	4.944	131.49	102.97	174.50	−179.8
2	4.998	127.92	179.28	115.87	−179.9
2′	4.946	130.17	179.22	111.16	−179.9
3	4.991	127.84	179.53	115.85	0.0
3′	4.939	130.30	179.31	111.17	−0.3
4	5.001	126.78	103.10	179.94	180.0
4′	4.964	129.65	103.25	174.31	−179.9
5	5.008	126.14	179.22	115.79	180.0
5′	4.961	128.11	179.08	111.02	180.0
6	5.002	125.96	179.62	115.93	0.0
6′	4.955	128.07	179.45	111.31	−0.2
			separation at 8 Å		
1	8.003	128.59	107.93	179.81	−178.5
1′	7.961	131.49	108.10	174.17	−178.3
2	8.004	127.90	179.43	115.89	−179.9
2′	7.952	130.18	179.34	111.14	−180.0
3	8.002	127.97	177.65	115.96	3.0
3′	7.949	130.39	177.48	111.25	2.5
4	8.012	126.69	108.01	179.90	−178.7
4′	7.918	130.56	108.43	174.42	179.7
5	8.015	125.99	179.39	115.83	180.0
5′	7.968	127.90	179.24	115.05	180.0
6	8.018	126.04	177.37	115.29	2.4
6′	7.965	128.11	177.50	111.34	2.4

complex as observed in our preliminary study. In the imidazolate−acetic acid dyad structures optimized using the two methods, the O−H bond length of the acid is 1.0 Å; the intermolecular hydrogen bond length of the dyad is 1.7 Å; and the hydrogen bond angle of NHO is 177 and 178° of arc, when using the B3LYP and MP2 theories, respectively. The distance between the hydrogen bond donor (O) and acceptor (N) in the imidazolate−acetic acid structures optimized with the two methods is 2.7 Å and agrees with the corresponding average distance (2.8 ± 0.4 Å) obtained by others from a survey of the three-dimensional structures of proteins containing carboxylate−histidine−zinc in the PDB.[4] The hydrogen bond angle of ONC in the optimized structures is 116 and 113° of arc when using the MP2 and B3LYP theories, respectively, which also agrees with the corresponding average angle of 116 ± 19° or arc obtained from the survey.[4] The coordinates of the optimized imidazolate−acetic acid dyad structures are provided in the Supporting Information.

To confirm the proton transfer from the imidazole to the carboxylate group in the presence of the zinc divalent cation, we also examined a model system in which one zinc divalent cation coordinates to two imidazole−formate dyads (Figure 2). The use of formate rather than acetate was to make the optimization of the bis dyad with the B3LYP/6-311+G(d,p) method feasible. Consistently, we found that the two imidazole−formate dyads linked with a zinc divalent cation converged to the two imidazolate−formic acid dyads bridged with a zinc ion that are shown in Figure 2 after a one month energy minimization, with the B3LYP/6-311+G(d,p) method performed on a dedicated SGI Origin 2000 computer with eight R10K processors. In the optimized imidazolate−formic acid dyad, the O−H bond length of the acid is 1.0 Å; the intermolecular hydrogen bond length of the dyad is 1.7 Å; and the hydrogen bond angle of NHO is 176° of arc. The distance between the hydrogen bond donor (O) and acceptor (N) is 2.7 Å and agrees with the corresponding experimentally observed average distance of 2.8 ± 0.4 Å.[4] The hydrogen bond angle of ONC is 115° of arc and also agrees with the corresponding experimentally observed

TABLE 3: Bond Lengths (Å), Angles (deg of arc), and Torsions (deg of arc) of the B3LYP/6-311+G(2d,2p)-Minimized Structures of Acetate, Acetic Acid, Imidazole Coordinating and Not Coordinating to the Hydroxide-Bound Zinc Divalent Cation, and Imidazolate Coordinating and Not Coordinating to the Hydroxide-Bound Zinc Divalent Cation Shown in Figure 1

	CH_3COOH	CH_3COO^-
CC^2	1.502	1.560
C^2O	1.204	1.255
C^2O^1	1.359	1.255
O^1H^1	0.968	
CH	1.090	1.095
CC^2O	126.1	116.2
OC^2O^1	122.3	128.8
$H^1O^1C^2$	106.8	
HCC^2	109.6	116.7
HCC^2O	0	6.47
$H^1O^1C^2O$	0	
	imidazole	imidazolate
N^1C^1	1.3639	1.3474
NC^1	1.3110	1.3474
N^1H^1	1.0050	
C^3H^3	1.0760	1.0819
C^4H^4	1.074	1.082
C^3N	1.376	1.370
C^3C^4	1.368	1.389
N^1C^4	1.378	1.370
HC^1	1.077	1.083
NC^1N^1	111.5	116.3
$C^1N^1C^4$	107.2	102.7
$N^1C^4C^3$	105.2	109.2
C^4C^3N	110.5	109.2
HC^1N^1	122.5	121.9
$H^1N^1C^4$	126.3	
$H^4C^4C^3$	132.5	129.1
H^3C^3N	121.5	121.7
$N^1C^4C^3N$	0	0
$HC^1N^1C^4$	180.0	180.0
$H^1N^1C^4H^4$	0	
$H^4C^4C^3H^3$	0	0
$H^3C^3NC^1$	180.0	180.0
	$OH^--Zn^{2+}-$imidazole	$OH^--Zn^{2+}-$imidazolate
N^1C^1	1.3351	1.3104
NC^1	1.3348	1.3743
N^1H^1	1.0092	
C^3H^3	1.0746	1.0759
C^4H^4	1.0741	1.0765
C^3N	1.3909	1.3893
OH^5	0.9622	0.9601
OZn	1.7485	1.7698
NZn	1.8966	1.8415
C^3C^4	1.3572	1.3661
N^1C^4	1.3778	1.3763
HC^1	1.0759	1.0785
H^5OZn	117.8	115.9
OZnN	172.6	175.5
$ZnNC^1$	126.7	127.4
NC^1N^1	109.2	113.1
$C^1N^1C^4$	109.3	105.2
$N^1C^4C^3$	106.0	110.2
C^4C^3N	108.3	106.7
HC^1N^1	124.5	124.7
$H^1N^1C^4$	125.8	
$H^4C^4C^3$	134.3	128.3
H^3C^3N	122.5	122.2
H^5ONC^1	−87.1	−92.5
$ZnNC^3C^1$	−179.3	179.1
$OZnNC^1$	−82.3	−90.9
$C^1N^1C^4C^3$	0.04	0
$N^1C^4C^3N$	0	0
$HC^1N^1C^4$	179.8	179.9
$H^1N^1C^4H^4$	−0.2	
$H^4C^4C^3H^3$	0.2	0.1
$H^3C^3NC^1$	179.9	179.9

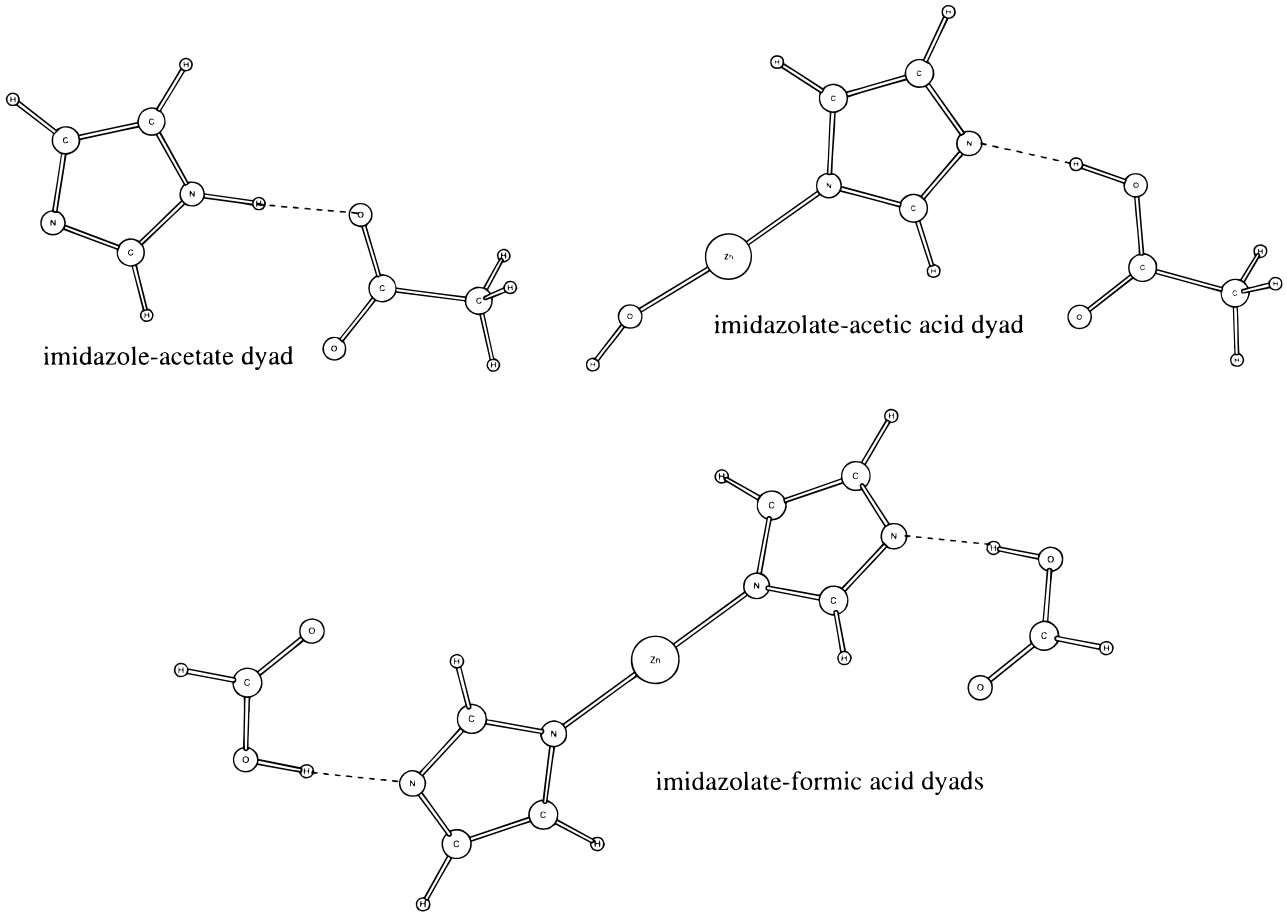

Figure 2. The imidazolate−acetic acid dyad, the imidazolate−formic acid dyad, and the imidazole−acetate dyad derived from energy minimizations with the B3LYP/6-311+G(d,p) method.

TABLE 4: Single-Point Energies (kcal/mol) of the Dyads Shown in Figure 1

	B3LYP/6-311+G(d,p)		MP2/6-311+G(d,p)	
system	$N^1-O^1 = 5$ Å	$N^1-O^1 = 8$ Å	$N^1-O^1 = 5$ Å	$N^1-O^1 = 8$ Å
1	−285 454.7	−285 448.4	−284 674.4	−284 667.7
1′	−285 442.4	−285 440.8	−284 667.9	−284 665.8
2	−285 452.3	−285 447.7	−284 671.8	−284 667.0
2′	−285 442.0	−285 441.3	−284 667.0	−284 666.1
3	−285 452.2	−285 447.7	−284 671.8	−284 667.0
3′	−285 442.0	−285 441.3	−284 667.0	−284 666.1
4	−1 449 540.7	−1 449 523.0	−1 447 874.6	−1 447 852.0
4′	−1 449 594.1	−1 449 593.5	−1 447 933.6	−1 447 932.6
5	−1 449 533.4	*a*	−1 447 865.7	−1 447 848.3
5′	−1 449 593.6	−1 449 593.5	−1 447 932.9	−1 447 932.6
6	−1 449 533.7	−1 449 521.0	−1 447 865.9	−1 447 848.4
6′	−1 449 593.5	−1 449 593.5	−1 447 932.8	−1 447 932.6

[a] not calculated due to the convergence problem.

average angle of 116 ± 19° of arc.[4] Using the MP2/LANL2DZ method, we also observed that the two imidazole−formate dyads linked with a zinc divalent cation converged to the two imidazolate−formic acid dyads bridged with a zinc ion shown in Figure 2. The use of the MP2/LANL2DZ method was to make the optimization of the bis dyad using the MP2 theory feasible. The coordinates of the optimized imidazolate−formic acid dyad structures are provided in the Supporting Information.

Most importantly, the above calculations using the B3LYP and MP2 theories suggest that the carboxylate group of Asp and Glu at the second coordination shell of a zinc complex is not a hydrogen bond acceptor[4] but rather a proton acceptor for the zinc-coordinated imidazole in proteins. Although more computationally intensive investigations of proton transfer between imidazole and acetate within a protein are required, the results reported here are consistent with our previously reported hypothesis of imidazolate as a zinc coordinate in proteins on the basis of ab initio calculations.[31]

Conclusions

The present ab initio calculations reveal that the imidazole−acetate dyad is less energetically stable than the imidazolate−acetic acid dyad in the presence of the zinc divalent cation and vice versa in the absence of zinc. The results suggest that the carboxylate group of Asp(Glu) at the second coordination shell of the zinc complex in proteins is not a hydrogen bond acceptor but rather a proton acceptor for the zinc-coordinated imidazole. Although more computationally intensive investigations of proton transfer between imidazole and acetate within a protein are required, the results reported here are consistent with our previously reported hypothesis of imidazolate as a zinc coordinate in proteins on the basis of ab initio calculations.

Supporting Information Available: The Cartesian coordinates of the imidazolate−acetic acid dyad, the imidazolate−formic acid dyad, and the imidazole−acetate dyad derived from energy minimizations with the MP2 and B3LYP theories, respectively. This material is available free of charge via the Internet at http://pubs.acs.org.

Acknowledgment. This work was supported by the Mayo Foundation and, in part, by the Summer Undergraduate Research Fellowship (to R.R.R.) from the Bonner Foundation.

References and Notes

(1) Nakagawa, S.; Umeyama, H.; Kitaura, K.; Morokuma, K. *Chem. Pharm. Bull.* **1981**, *29*, 1.

(2) Eklund, H.; C.-I., B. The Role of Zinc in Alcohol Dehydrogenase. In *Zinc Enzymes*; Spiro, T. G., Ed.; John Wiley & Sons: New York, 1983; pp 124−153.

(3) Laurenczy, G.; Ducommun, Y.; Merbach, A. E. *Inorg. Chem.* **1989**, *28*, 3024−3028.

(4) Christianson, D. W.; Alexander, R. S. *J. Am. Chem. Soc.* **1989**, *111*, 6412−6419.

(5) Vallee, B. L.; Auld, D. S. *Biochemistry* **1990**, *29*, 5647−5659.

(6) Vedani, A.; Huhta, D. W. *J. Am. Chem. Soc.* **1990**, *112*, 4759−4767.

(7) Hoops, S. C.; Anderson, K. W.; Merz, K. M. J. *J. Am. Chem. Soc.* **1991**, *113*, 8262−8270.

(8) Christianson, D. W. *Adv. Protein Chem.* **1991**, *42*, 281−355.

(9) Garmer, D. R.; Krauss, M. *J. Am. Chem. Soc.* **1993**, *115*, 10 247−10 257.

(10) Christianson, D. W.; Khoury-Christianson, A. M. Architecture and Design of Zinc Protein−Ligand Complexes. In *Chemical and Structural Approaches to Rational Drug Design*; Weiner, D. B., Williams, W. V., Eds.; CRC Press: Boca Raton, 1995; pp 215−236.

(11) Ryde, U. *Proteins* **1995**, *21*, 40−56.

(12) Stote, R. H.; Karplus, M. *Proteins* **1995**, *23*, 12−31.

(13) Ryde, U. *Eur. Biophys. J.* **1996**, *24*, 213−221.

(14) Berg, J. M.; Shi, Y. *Science* **1996**, *271*, 1081−1085.

(15) Lipscomb, W. N.; Strater, N. *Chem. Rev.* **1996**, *96*, 2375−2433.

(16) Wasserman, Z. R.; Hodge, C. N. *Proteins* **1996**, *24*, 227−237.

(17) Garmer, D. R. *J. Phys. Chem. B* **1997**, *101*, 2945−2953.

(18) Alberts, I. L.; Nadassy, K.; Wodak, S. J. *Protein Sci.* **1998**, *7*, 1700−1716.

(19) Fu, H. W.; Beese, L. S.; Casey, P. J. *Biochemistry* **1998**, *37*, 4465−4472.

(20) Lu, D. S.; Voth, G. A. *Proteins* **1998**, *33*, 119−134.

(21) Lu, D. S.; Voth, G. A. *J. Am. Chem. Soc.* **1998**, *120*, 4006−4014.

(22) Pavlov, M.; Siegbahn, P. E. M.; Sandstrom, M. *J. Phys. Chem.* **1998**, *102*, 219−228.

(23) Garmer, D. R.; Gresh, N.; Roques, B. P. *Proteins* **1998**, *31*, 42−60.

(24) Yamamura, T.; Nakamura, H.; Nakajima, S.; Sasaki, T.; Ushiyama, M.; Ueki, M.; Hirota, H. *Inorganica Chimica Acta* **1998**, *283*, 243−250.

(25) Roe, R. R.; Pang, Y. P. *J. Mol. Model.* **1999**, *5*, 134−140.

(26) Vaz, R. J.; Kuntz, I. D.; Meng, E. C. *Med. Chem. Res.* **1999**, *9*, 479−489.

(27) Zhan, C. G.; de Souza, O. N.; Rittenhouse, R.; Ornstein, R. L. *J. Am. Chem. Soc.* **1999**, *121*, 7279−7282.

(28) Toba, S.; Colombo, G.; Merz, K. M. *J. Am. Chem. Soc.* **1999**, *121*, 2290−2302.

(29) Xu, K.; Perola, E.; Prendergast, F. G.; Pang, Y.-P. *J. Mol. Model.* **1999**, *5*, 203−217.

(30) Pang, Y.-P. *J. Mol. Model.* **1999**, *5*, 196−202.

(31) ElYazal, J.; Pang, Y.-P. *J. Phys. Chem. B* **1999**, *103*, 8773−8779.

(32) Bernstein, F. C.; Koetzle, T. F.; Williams, G. J.; Meyer, E. E., Jr.; Brice, M. D.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. *J. Mol. Biol.* **1977**, *112*, 535−42.

(33) Frisch, M. J.; Trucks, G. W.; H. B., S.; Gill, P. M. W.; Hohnson, B. G.; Robb, M. A.; Raghavachari, K.; Al-Laham, M. A.; Zakrzewski, V. G.; Ortiz, J. V.; Foresman, J. B.; Cioslowski, J.; Stefanov, B. B.; Nanayakkara, A.; Challacombe, M.; Peng, C. Y.; Ayala, P. Y.; Chen, W.; Wong, M. W.; Andres, J. L.; Replogle, E. S.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Binkley, J. S.; Defrees, D. J.; Baker, J.; Stewart, J. P.; Head-Gordon, M.; Gonzales, C.; Pople, J. A. *Gaussian, Inc.*, Pittsburgh, PA **1999**.

(34) Møller, C.; Plesset, M. S. *Phys. Rev.* **1934**, *46*, 618.

(35) Head-Gordon, M.; Pople, J. A.; Frisch, M. J. *Chem. Phys. Lett.* **1988**, *153*, 503.

(36) Frisch, M. J.; Head-Gordon, M.; Pople, J. A. *Chem. Phys. Lett.* **1990**, *166*, 275.

(37) Frisch, M. J.; Head-Gordon, M.; Pople, J. A. *Chem. Phys. Lett.* **1990**, *166*, 281.

(38) Head-Gordon, M.; Head-Gordon, T. *Chem. Phys. Lett.* **1994**, *220*, 122.

(39) Saebo, S.; Almlof, J. *Chem. Phys. Lett.* **1989**, *154*, 83.

(40) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648−5652.

(41) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev.* **1988**, *B37*, 785.

(42) Hehre, W. J.; Radom, L.; Schleyer, P. V. R.; Pople, J. *Ab Initio Molecular Orbital Theory*; Wiley: New York, 1986.

(43) Hay, P. Y.; Wadt, W. R. *J. Chem. Phys.* **1985**, *82*, 299.

(44) Bacskay, G. B. *Chem. Phys.* **1981**, *61*, 385−404.

(45) QUANTA/CHARMm. *San Diego, CA* **1997**.

(46) Han, W. G.; Suhai, S. *J. Phys. Chem.* **1996**, *100*, 3942−3949.

(47) Li, G. S.; Maigret, B.; Rinaldi, D.; Ruizlopez, M. F. *J. Comput. Chem.* **1998**, *19*, 1675−1688.

(48) Lias, S. G.; Liebman, J. E.; Levin, R. D. *J. Phys. Chem. Ref. Data* **1984**, *13*, 695.