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A Density Functional Study of the Receptor−Ligand Interaction: Stabilization Energy in Ammonium Salt−Aromatic Interactions

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Amino−aromatic interactions have been well examined in the fields of protein structure determination and theoretical calculation. Under physiological conditions, however, most amines in proteins exist as ammonium salts, and ammonium salt−aromatic interactions are supposed to be more predominant in protein structures than amino−aromatic interactions. A nonlocal density functional theory molecular orbital calculation was applied to an ammonia−benzene system and gave an interaction energy of 0.9 kcal/mol, which was in good agreement with an experimental value (1.4 kcal/mol). A series of calculations also revealed that the interaction energy was in the range 2.5−6.2 kcal/mol for several ammonium formate−aromatic systems, which could play as important a role in receptor−ligand interactions as hydrogen bonding (4.5−6.1 kcal/mol) does.

Introduction

Amino−aromatic interactions were first observed in structures of haemoglobin−drug complexes.[1] Burley and Petsko surveyed a number of X-ray structures to demonstrate a marked tendency for buried amino groups in proteins to be found above or below the center of aromatic rings.[2] The suggestion by Levitt and Perutz[3] that aromatic rings could act as hydrogen-bond acceptors in proteins implies the formation of significant attractive interactions between proton donors such as amino groups and aromatic rings. The most typical example was found in the crystal structure of the phosphotyrosine recognition domain SH2 of *v-src* complexed with tyrosine-phosphorylated peptides.[4] Ab initio molecular orbital calculations with MP2/6-31G** basis sets provided an interaction energy of an ammonia−benzene system to be about 2.4 kcal/mol.[5] Under physiological conditions, however, most amino groups in protein structures exist as ammonium forms with their counteranions and act as stronger acids than amines. Therefore, ammonium salt−aromatic interactions should contribute more dominantly than amino−aromatic ones to stabilizing protein structures. Such ammonium salt−aromatic interactions were studied also by Kim's group.[6] Extensive conformational analyses of these kinds of interactions have recently been reported on the basis of X-ray crystal structures by Thornton el al.[7]and by Dougherty.[8] Although the ammonium salt−aromatic interaction was studied with ab initio calculations (5−10 kcal/mol with STO-3G basis sets and 14−17 kcal/mol with 3-21G basis sets),[9] it has turned out that higher basis sets and a perturbation procedure should be employed for precise evaluation of relatively small energies induced by intermolecular interactions. As such a heavy calculation requires a tremendous amount of computation, alternative ways have been sought by computational chemists, especially those who have to handle bigger systems such as pharmaceutical drugs. Density functional theory (DFT)[10] molecular orbital calculation methods have been applied to these systems because they need less computer resources than ordinary molecular orbital calculations, without loss of accuracy. We herein evaluate the validity of DFT calculations applied to the ammonia−benzene system[5] and estimate interaction energies to ammonium salt−aromatic systems as well.

Experimental Section

DFT Calculations. DMol[11] version 2.35 and 2.36 on an IRIS Indigo workstation (Silicon Graphics Inc.) was used for all DFT calculations. To ensure the accuracy of the calculations, we employed a double numerical plus polarization (DNP) basis set with a FINE mesh, which is thought to be equivalent in precision to MP2 or MP4 calculation,[10] and a nonlocal spin density (NLSD) method to take into account subtle interactions such as hydrogen bonding. Becke's 1988 version of a gradient-corrected exchange functional[12] and a Lee−Yang−Parr correction functional[13] were also used without frozen core.

Model Building. The MP2/6-31G** optimized structure of the ammonia−benzene complex[5] was used as an initial model. This "monodentate" structure shown in Figure 1a was optimized using a BFGS optimizer with a damping factor 0.4 for gradients, until each absolute gradient value reached less than 0.000 05. The "bidentate" structure shown in Figure 1b was built such that the distance from two hydrogens of ammonia to the plane of the benzene ring was equally set to 2.5 Å. The interaction energy was calculated by subtracting the sum of the total energies for ammonia and benzene from the total energy of the whole complex.

The initial models of ammonium formate−aromatic systems were constructed from the optimized "monodentate" complex of ammonia−benzene described above. The ammonia moiety was converted into ammonium cation by substituting the lone pair with a hydrogen. A formate anion was then located over one of the hydrogens of the benzene ring with its plane parallel to the benzene ring as shown in Figure 1c. The distances from the formate oxygens to the ammonium nitrogen were set equal to 2.8 Å initially. Finally, all the unique configurations of the ternary complex having different orientations of the formate

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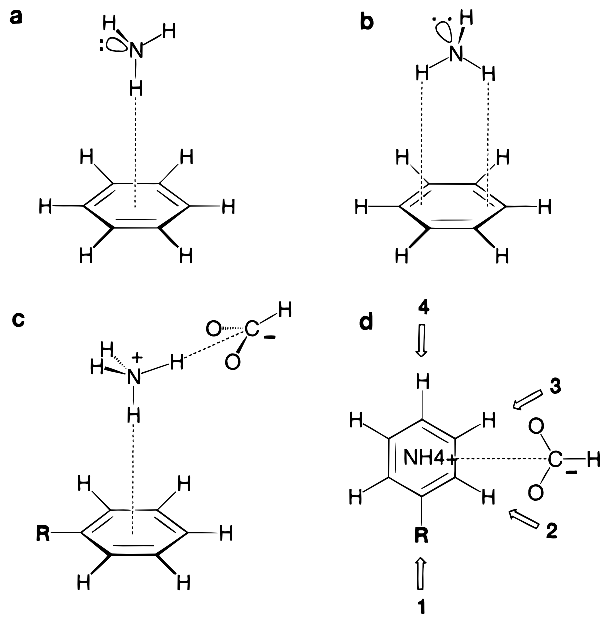

Figure 1. Schematic drawings of the initial structures. The initial models for “monodentate” and “bidentate” forms of ammonia−benzene complexes are schematically drawn in the top row. In both models, the nitrogen atom of the ammonia was located above the center of the benzene ring. The hydrogen toward the benzene molecule was on an axis between the nitrogen and the center of the benzene ring in the “monodentate” complex (a), while two hydrogens of the ammonia were equally bifurcated by this axis at distances of 2.5 Å to the benzene ring in the bidentate complex (b). The starting structure of the ammonium salt−benzene complex is also illustrated in the lower row. The model was built from the “monodentate” form of the ammonia−benzene complex by adding an extra hydrogen atom to the ammonia and by adding a formate anion to form a salt bridge with the ammonia (c). For substituted benzenes and heterocyclic rings, the relative orientation of the formate with respect to the position of the substituent or heteroatom was positioned as if the formate were to be the virtual substituent of the aromatic system (d).

moiety relative to the substituted group were generated for each substituted aromatic system. Figure 1d shows the definition of the relative orientation of the formate. Each structure was then optimized as shown above until each absolute gradient value reached less than 0.0001. During each minimization process, the position of the ammonium hydrogen facing the formate was carefully monitored to prevent its migration toward the formate. The calculation was simply abandoned when the situation above was detected.

Results and Discussion

Ammonia−Aromatic Interactions. Rodham et al. studied the interaction energy of ammonia−benzene.[5] They reported the interaction energy of 1.4 kcal/mol measured by experiment, and MP2/6-31G** calculations provided 2.4 kcal/mol for the “monodentate” structure.[5] In this kind of nonbonded interaction, ammonia acts as a proton donor and benzene as an acceptor.[5] We applied DFT calculations for this system to evaluate its validity. The interaction energy obtained was 0.9 kcal/mol for the “monodentate” structure, which was much closer to the experimental value than that from the MP2/6-31G** calculation. The distance along the normal line from the amino nitrogen to the benzene ring was 3.685 Å from our DFT, which was also in better agreement with the experimentally determined value (3.59 Å) than that from the MP2 result (3.43 Å).[5] The benzene ring in the monodentate complex was slightly deformed, like a dish, such that the least-squares plane of all hydrogens was displaced by 0.008 Å toward the ammonia relative to benzene alone. These results strongly support that DFT is applicable to the calculation of such subtle intermolecular interactions. The interaction energy for the “bidentate” structure, on the other hand, was 0.1 kcal/mol, which would presumably not contribute much to the stabilization of the system.

TABLE 1: Interaction Energies for Ammonium Formate−Aromatic Systems

aromatic system	orientation[a]	energy[b] (kcal/mol)	Ca−N[c] (Å)	Ca−H[d] (Å)
benzene		4.06	3.642	2.597
chlorobenzene	3	2.49	3.646	2.602
fluorobenzene	2	2.71	3.640	2.596
	4	3.35	3.634	2.591
toluene	3	3.14	3.636	2.592
phenol	1	3.59	3.675	2.630
	2	3.34	3.647	2.604
	3	5.11	3.642	2.597
	4	3.83	3.652	2.605
anisole	2	6.22	3.577	2.531
indole	1	5.79	3.625	2.579
	5	4.43	3.593	2.549
	6	4.77	3.615	2.570
	7	6.12	3.623	2.578
imidazole	3	5.75	3.621	2.576

[a] Relative formate orientation to the substituent or heteroatom of the aromatic system (see in Figure1d). [b] Interaction energy. [c] Distance from N of the ammonium to the aromatic plane. [d] Distance from the proximal H of the ammonium to the aromatic plane.

Ammonium Formate−Aromatic Interactions. Interaction energies in ammonium formate−aromatic systems were calculated using typical aromatic compounds such as benzene, phenol, imidazole, and indole, which are found in side chains of proteins, as well as other substituted benzenes. Because ammonium cations were found over the top of aromatic rings in most protein structures,[6] the optimized geometry of the ammonia−benzene described above was employed as a starting model. With the exception of unsubstituted benzene, the anisotropic arrangement of the formate anion with respect to the asymmetric molecular shape of an aromatic compound should be taken into account. Therefore, possible starting models with different geometries were generated, in each of which the formate is oriented differently with respect to the substituent of benzene or to the heteroatom in imidazole and indole. It turned out from the subsequent DFT calculations that only a few starting models could successfully be optimized among all the possible orientations of formate relative to anisotropic aromatic rings (Table 1). Otherwise, a hydrogen of the ammonium cation was abstracted by the formate to form a neutral ammonia−formic acid complex, or some gradients of coordinates could not reach below 0.0001. The ammonium salt−aromatic interaction is solely influenced by the electrostatic property of the aromatic ring due to substituents or heteroatoms. It is reasonable that electron-withdrawing substituents reduce the interaction energy whereas electron-donating groups stimulate it, since aromatic rings act as weak hydrogen acceptors in these complexes.[5] The interaction energy is weakly correlated with the distance from either the stacked hydrogen or the nitrogen ammonium to the aromatic ring (correlation coefficients of 0.61 and 0.63). This phenomenon was also seen in CH-π interactions[14] and Na-π interactions.[15]

Deakyne and Meot-Ner reported that the ammonium salt−aromatic interaction was in the range 5−10 kcal/mol using STO-3G basis sets and 14−17 kcal/mol using 3-21G basis sets.[8] From the assumption that our nonlocal DFT calculation provided more reasonable estimates than MP2 for this system as well as that for the ammonia−benzene system, the values from STO-3G and 3-21G calculations[11] would be overestimated due to the usage of oversimplified basis sets and calculation methods. Although Caldwell and Kollman also reported that the ammonium−benzene interaction was 19.1 kcal/mol using MP2/6-31G*//MP2/6-31G*, the minimum-energy distance from the "center" of the ion to the center of the benzene ring was 2.91 Å,[16] which seems too short compared with the experimental distance between the ammonia nitrogen and the center of the benzene ring (3.59 Å). Our calculations imply side chains of aromatic amino acid residues (Phe, Try, His, Trp) stabilize the ammonium salts in protein structures by 4−6 kcal/mol, the same magnitude as hydrogen bonding (4.5−6.1 kcal/mol).[17] The marked orientational preference of the ammonium salt−aromatic interaction gives us a means to predict the binding mode of ligands to their receptor proteins.[18]

Conclusion

The amine−aromatic interaction was examined with the nonlocal DFT molecular orbital calculation. The geometrical and energetic properties of the amine−aromatic complex were in good agreement with those found in experimental measurements. The accuracy of the calculation was virtually equal to (or even slightly better than) that of the ordinary ab initio molecular orbital calculations, which usually require much more computational resources. As the aim to prove the capability of the DFT calculation was satisfactorily met, the same procedure was applied to a series of ammonium formate−aromatic complexes in order to estimate the energies of ammonium salt−aromatic interactions. The calculations revealed that the interaction energies were as strong as those of hydrogen bonding. We conclude that the DFT method is a promising one for molecular complex systems in which weak intermolecular interactions occur and is capable of reproducing reliable geometrical and energetic properties with moderate computer resources.

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Supporting Information Available: Listing of the coordinates for the complexes (15 pages). See any current masthead page for ordering and Internet access instructions.

References and Notes

(1) Perutz, M. F.; Fermi, G.; Abraham, D. J.; Poyart, C.; Bursaux, E. *J. Am. Chem. Soc.* **1986**, *108*, 1064−1078.

(2) Burley, S. K.; Petsko, G. A. *FEBS Lett.* **1986**, *203*, 139−143.

(3) Levitt, M.; Perutz, M. F. *J. Mol. Biol.* **1988**, *201*, 751−754.

(4) Waksman, G.; Kominos, D.; Robertson, S. C.; Pant, N.; Baltimore, D.; Birge, R. B.; Cowburn, D.; Hanafusa, H.; Mayer, B. J.; Overduin, M.; Resh, M. D.; Rios, C. D.; Silverman, L.; Kuriyan J. *Nature* **1992**, *358*, 646−653.

(5) Rodham, D. A.; Suzuki, S.; Suenram, R. D.; Lovas, F. J.; Dasgupta, S.; Goddard III, W. A.; Blake, G. A. *Nature* **1993**, *362*, 735−737.

(6) Kim, K. S.; Lee, J. Y.; Lee, S. J.; Ha, T. K.; Kim, D. H. *J. Am. Chem. Soc.* **1994**, *116*, 7399−7400. Lee, J. Y.; Lee, S. J.; Choi, H. S.; Cho, S. J.; Kim, K. S.; Ha, T. K. *Chem. Phys. Lett.* **1995**, *232*, 67−71.

(7) Flocco, M. M.; Mowbray, S. L. *J. Mol. Biol.* **1994**, *235*, 709−717. Mitchell, J. B. O.; Nandi, C. L.; McDonald, I. K.; Thornton, J. M.; Price, S. L. *J. Mol. Biol.* **1994**, *923*, 315−331.

(8) Dougherty, D. A. *Science* **1996**, *271*, 163−168.

(9) Deakyne, C. A.; Meot-Ner, M. *J. Am. Chem. Soc.* **1985**, *107*, 474−479.

(10) *Density Functional Methods in Chemistry*; Labanowski, J., Andzelm, J., Eds.; Springer-Verlag: New York, 1991.

(11) Molecular Simulations Inc., San Diego, CA.

(12) Becke, A. D. *J. Chem. Phys.* **1988**, *88*, 2547−2553.

(13) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev.* **1988**, *B37*, 786−789.

(14) Paliwal, S. G.; Wilcox, C. S. *J. Am. Chem. Soc.* **1994**, *116*, 4497−4498. Nishio, M.; Umezawa, Y.; Hirota, M.; Takeuchi, Y. *Tetrahedron* **1995**, *51*, 8665−8701.

(15) Mecozzi, S.; West, A. P., Jr.; Dougherty, D. A. *J. Am. Chem. Soc.* **1996**, *118*, 2307−2308.

(16) Caldwell, J. W.; Kollman, P. A. *J. Am. Chem. Soc.* **1995**, *117*, 4177−4178.

(17) Joesten, M. D.; Schaad, L. J. *Hydrogen Bonding*; Dekker: New York, 1974.

(18) Yamano, Y.; Ohyama, K.; Kikyo, M.; Sano, T.; Nakagomi, Y.; Inoue, Y.; Nakamura, N.; Morishima, I.; Guo, D.-F.; Hamakubo, T.; Inagami, T. *J. Biol. Chem.* **1995**, *270*, 14024−14030.