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On the Structure of the Arginine-carboxylate Salt Bridge: A Density Functional Theory Study

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Abstract: High-level density functional theory (DFT) calculations are performed for the first time to answer the question whether the arginine-carboxylate salt bridge stays in a zwitterionic state or a neutral one. The results indicate that in the gas phase, the neutral form is more stable and hence proton transfer occurs from guanidinium to carboxylate. However, in an aqueous solution the zwitterionic form should be favored. The difference might be caused by the electrostatic interaction between the salt bridge and its molecular environment. Therefore, the solvation effect has to be considered in the modeling of proteins, whose stabilization depends heavily on the salt-bridges.

Keywords: Arginine-carboxylate interaction, salt bridge, DFT, proton transfer, solvation effect.

The arginine-carboxylate salt bridge, which represents about 40% of the pairs of ionic groups within proteins, plays a crucial role in determining the structures and functions of proteins[1]. Numerous experimental evidences have indicated that this type of salt bridge, which usually includes the arginine-glutamate pair and the arginine-aspartate pair, should stay in a zwitterionic state rather than in a neutral one[2]. This viewpoint has been widely accepted and in usual it is directly used in the theoretical studies on protein structures and enzyme mechanisms[3].

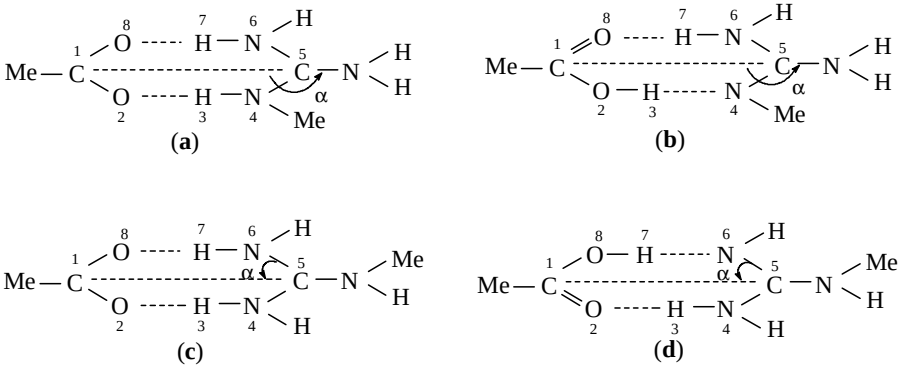
However, based on a recent calculation in vacuum at the level of AM1 and HF it was suspected that the arginine-carboxylate salt bridge should more likely stay in a neutral state than in a zwitterionic one[4]. Apparently, this controversial result will bring about many serious problems in the modeling of proteins if it is correct. Therefore, in the present study high-level density function theory calculations were performed for the first time to investigate the problem in depth. The methylguanidinium-acetate pair was selected as an appropriate model of the arginine-carboxylate salt bridge. Both the gas-phase interaction and the solvation effect were considered in detail, and it turns out that the stability of the zwitterionic form and its neutral counterpart depends heavily on the molecular environment sensed by the salt bridges.

Methods

All the calculations were performed with the GAUSSIAN 98 software[5]. Four forms of the methylguanidinium-acetate pair were considered, *i.e.* zwitterionic *trans* (H), neutral

trans (H), zwitterionic *trans* (CH_3), and neutral *trans* (CH_3) (See **Figure 1**). Density functional theory optimizations at the level of B3LYP/6-311G**[6] were performed on the four forms, respectively. No geometry constraints were put on the systems during the optimization. The solvation effect was taken into account by employing a continuum solvation model based on the self-consistent reaction field (SCRF) method, which has been shown recently to be an appropriate method for the study of ion pair interactions[7].

Figure 1 Schematic representation of the four forms of the methylguanidinium-acetate pair



(a) ZT(H), (b) NT(H), (c) ZT(CH_3), (d) NT(CH_3)

Results and Discussion

The total energies of the four forms of the methylguanidinium-acetate pair in the gas phase were listed in **Table 1**, and the corresponding structure parameters were summarized in **Table 2**.

Table 1 Total energies of the four forms of the methylguanidinium-acetate pair in vacuum

Form	ZT(H)	NT(H)	ZT(CH_3)	NT(CH_3)
E (kJ/mol)	-1243129.68	-1243136.23	-1243127.53	-1243139.51

From **Table 1**, it can be seen that in vacuum the neutral forms are always more stable than the corresponding zwitterionic ones. This result agrees with that by Melo *et al*[4], though here the calculation is at a much higher DFT level. The energy difference between the neutral form and the corresponding zwitterionic one is around 10 kJ/mol, which is obviously not negligible. Examination of the optimized structures turns out that from the neutral form to the zwitterionic counterpart, one O–H bond is significantly lengthened and one N–H bond is significantly shortened. In consequence, proton transfer occurs from guanidinium to carboxylate in the gas phase. Interestingly, from **Table 2** it can be seen that during the proton transfer the bond length of C1-C5 also significantly changes, and as a result the distance between guanidinium and carboxylate is always longer in the neutral forms than in the zwitterionic ones.

Table 2 Structure parameters of the four forms of the methylguanidinium-acetate pair

Species	α	Distance (Å)						
	(deg.)	C1-C5	O8-H7	H7-N6	O2-H3	H3-N4	O8-N6	O2-N4
ZT(H)	174.1	3.84	1.54	1.08	1.50	1.10	2.62	2.58
NT(H)	172.1	4.00	1.89	1.02	1.04	1.61	2.90	2.64
ZT(CH_3)	58.8	3.83	1.45	1.11	1.56	1.08	2.56	2.63
NT(CH_3)	54.3	4.00	1.03	1.62	1.89	1.02	2.65	2.90

The reason for the above proton transfer can be seen when the dipole moment of the methylguanidinium-acetate pair is considered. From B3LYP/6-311G** calculations, the dipole moments of the zwitterionic forms (7.65 and 8.17 Debye) are much larger than those of their neutral counterparts (3.54 and 4.07 Debye). Usually, a large separation of charge in vacuum is not favored. Therefore, in vacuum the neutral form should be more stable.

However, when the above calculations are re-performed in an aqueous solution, the result changes significantly. From **Table 3**, it can be seen that in water, the zwitterionic forms become more stable than the corresponding neutral ones. The energy difference between the two forms is again around 10 kJ/mol and hence, is significant. This result agrees with that by the experimentalists. Therefore, in proteins, the arginine-carboxylate salt bridges should be located in an environment that is closer to the aqueous solution than to the gas phase. Interestingly, although in vacuum the NT(CH_3) form is the most stable, it becomes the least favorable in the aqueous solution. All the above results indicate that the influence of solvation is very important to the relative stability of the different forms of the salt bridge.

Table 3 Total energies of the four forms of the methylguanidinium-acetate pair in water

Form	ZT(H)	NT(H)	ZT(CH_3)	NT(CH_3)
α_0 (Å)	4.35	4.42	4.49	4.38
E (kJ/mol)	-1243155.05	-1243141.32	-1243153.92	-1243146.59

Note: Here α_0 is the solute cavity radius. It was obtained from a gas-phase molecular VOLUME calculation using the Gaussian 98 software.

The reason for the above results from the SCRF calculation can be understood from the interaction between the solute and the solvent. As known, the SCRF solvation model considers the solvent as a continuous dielectric with a cavity accurately modeled for the solute. The solvent reacts against the solute charge distribution, generating a reaction field. The electrostatic interaction between the solute and the solvent is introduced as a perturbation operator in the solute hamiltonian. Herein, the solvent is water, and it is well known that while the interaction between water and ions is generally strong, the interaction between water and organic molecules is usually weak. Hence, it can be expected that the interaction between water and the zwitterionic form of the salt bridge is favorable, because in the latter significant charge separation can be sensed. However, the interaction between water and the neutral form of the salt bridge cannot be strong, because in the neutral form the salt bridge is essentially a complex of two organic molecules. As a result, the relative stability of the different forms of the salt bridge

depends heavily on the molecular environment they are sensing. Since the salt bridges play important roles in stabilizing the structures of proteins, it is strongly advised that in the modeling of proteins the solvation effect should always be considered.

Conclusion

High-level density functional theory calculations were performed on the arginine-carboxylate salt bridge. The results indicated that although the neutral form was favored in vacuum, the zwitterionic form was more stable in an aqueous solution. The electrostatic interaction between the salt bridge and its molecular environment was proposed to be the reason for the above behaviors. Therefore, consideration of the solvation effect is very important in the modeling of proteins.

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References

1. (a) J. F. Riordan, K. D. McElvany, C. L. Borders, *Science*, **1977**, *195*, 884. (b) D. J. Barlow, J. M. Thornton, *J. Mol. Biol*., **1983**, *168*, 867. (c) J. Singh, J. M. Thornton, M. Snarey, S. F. Campbell. *FEBS Lett*., **1987**, *224*, 161.
2. (a) C. H. Görbitz, *Acta Crystallogr. B*, **1989**, *45*, 390. (b) J. A. Ippolito, R. S. Alexander, D. W. Christianson, *J. Mol. Biol.*, **1990**, *215*, 457. (c) J. B. O. Mitchell, J. M. Thornton, J. Singh., *J. Mol. Biol.*, **1992**, *226*, 251. (d) P. Chakrabarti. *Int. J. Peptide Protein Res*., **1994**, *43*, 284.
3. (a) A. M. Sapse, C. S. Russell, *Int. J. Quantum Chem*., **1984**, *26*, 91. (b) A. M. Sapse, C. S. Russell, *J. Mol. Struct. (Theochem)*, **1986**, *137*, 43. (c) D. W. Deerfield, H. B. Nicholas Jr., R. G. Hiskey, L. G. Pedersen, *Proteins*, **1989**, *6*, 168. (d) J. B. O. Mitchell, C. L. Nandi, J. M. Thornton, S. L. Price, J. Singh, M. Snarey, *J. Chem. Soc. Faraday Trans*., **1993**, *89*, 2619.
4. (a) A. Melo, M. J. Ramos, *Chem. Phys. Lett*., **1995**, *245*, 498. (b) A. Melo, M. J. Ramos, W. B. Floriano, J. A. Gomes, J. F. Leao, A. L. Magalhaes, B. Maigret, M. C. Nascimento, N. Reuter, *J. Mol. Struct. (Theochem)*, **1999**, *463*, 81.
5. M. J. Frisch, G. W. Trucks, H. B. Schlegel, et al., *Gaussian 98*, Revision A. 7., Gaussian Inc., Pittsburgh PA, **1998**.
6. (a) A. D. Becke., *J. Chem. Phys*., **1993**, *98*, 5648. (b) C. Lee, W. Yang, R. G. Parr., *Phys. Rev. B*, **1988**, *37*, 785.
7. (a) K. Y. Nam, J. H. Yoon, K. T. No., *Chem. Phys. Lett*., **2000**, *319*, 391. (b) K. H. Cho, K. T. No, H. A. Scheraga, *J. Phys. Chem. A*, **2000**, *104*, 6505.

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