Influence of a Hydrophobic Environment on the Structure of Arginine-Carboxylate Salt Bridge

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The exact structure of an arginine-carboxylate salt bridge in different chemical environments remains a controversial problem. In the present work, the zwitterionic and neutral forms of arginine-carboxylate salt bridge were studied by the B3LYP/6-311G(d,p)//PM3 method. It turns out that the neutral forms are more stable than the zwitterionic counterparts in gas phase. However, when bound by α-cyclodextrin, the zwitterionic forms become more stable than the corresponding neutral ones. It is suggested that the hydrophobic environment provided by the cyclodextrin cavity leads to such behavior. Therefore, the salt bridge still could be in a zwitterionic form in the hydrophobic interior of the real proteins.

Keywords arginine-carboxylate interaction, salt bridge, cyclodextrin, PM3, DFT

Introduction

The arginine-carboxylate salt bridge, which represents about 40% of the pairs of ionic groups in proteins, plays a crucial role in determining the structures and functions of proteins.[1] Numerous experimental evidences collected indicate that this type of salt bridge, which usually includes the arginine-glutamate pair and the arginine-aspartate pair, should stay in a zwitterionic form rather than in a neutral one.[2] However, based on a theoretical calculation *in vacuo* it was recently suspected that the arginine-carboxylate salt bridge should more likely stay in a neutral form than in a zwitterionic one.[3] Apparently, this result might bring about serious problems, because a zwitterionic form of arginine-carboxylate salt bridge has been routinely assumed in the theoretical studies on protein structures and enzyme mechanisms.[4]

In fact, it is known that ion pairs such as ammonium carboxylates (including amino acid zwitterions) are unstable in gas phase, which revert to the thermodynamically favored amine-carboxylic acid complexes.[5] On the other hand, it has been demonstrated that polar solvent molecules (particularly water) can significantly stabilize the zwitterionic salt bridges.[6] For instance, using a continuum solvation model, we have recently shown that the zwitterionic form of arginine-carboxylate salt bridge is strongly favored over the neutral one in aqueous solution.[7] Thus, the stability of the zwitterionic arginine-carboxylate salt bridge might be strongly dependent on its chemical environments. It seems impossible that an arginine-carboxylate pair buried in a hydrophobic internal cavity of protein is a zwitterion.

Herein, the possible influence of a hydrophobic environment on the structure of arginine-carboxylate salt bridge with quantum chemistry methods was investigated. Because of its large size, a real protein system is obviously not an appropriate choice. Therefore, the structure of an arginine-carboxylate salt bridge buried in an artificial host molecule, α-cyclodextrin (α-CD), was studied. This molecule has a well-defined hydrophobic internal cavity, and its inclusion complexation has been shown to be able to mimic protein-substrate interactions.[8]

Methods

All the calculations were performed with GAUSSIAN 98.[9] The methylguanidinium-acetate pair was selected as an appropriate model of the arginine-carboxylate salt

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bridge. Four forms of the methylguanidinium-acetate pair were considered, *i.e.* zwitterionic *trans*(H)[ZT(H)], neutral *trans*(H)[NT(H)], zwitterionic *trans*(CH_3)[ZT(CH_3)], and neutral *trans*(CH_3)[NT(CH_3)] (Fig. 1). They were optimized by PM3 method *in vacuo* and in α-CD cavity.

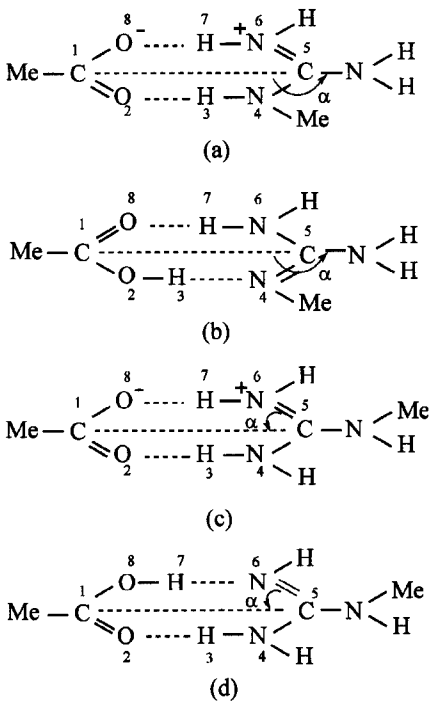

Fig. 1 Schematic representation of the four forms of the methylguanidinium-acetate pair: (a) ZT(H), (b) NT(H), (c) ZT(CH_3) and (d) NT(CH_3).

The complexation of the salt bridge with α-CD was simulated by putting the guest in one end of CD and then letting it pass through the CD cavity by steps.[10] (Fig. 2) In particular, the glycosidic oxygens were placed onto the *XY* plane and their center was defined as the center of the coordination system. The primary OH groups were placed pointing toward the positive *Z*-axis. The inclusion complex was constructed from the PM3-optimized α-CD and salt bridge. The longer dimension of the substrate was initially placed onto the *Z*-axis. Its position was defined by the *Z* coordinate of C(1) of the salt bridge. The inclusion complexation was emulated by entering substrate from one end of α-CD and then letting it pass through α-CD by steps. In every step, the geometry of the host-guest complex was completely optimized by PM3 without any restriction. Finally, DFT single-point calculation at the level of B3LYP/6-311G(d,p) was performed on all the PM3-optimized species.

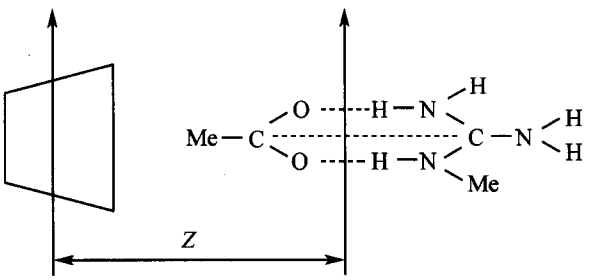

Fig. 2 Relative position of the salt-bridge to α-CD.

Results and discussion

The total energies of the four forms of the methylguanidinium-acetate pair calculated with B3LYP/6-311G(d,p)//PM3 method *in vacuo* are listed in Table 1, and the corresponding structure parameters are summarized in Table 2. From Table 1, it can be seen that *in vacuo* the neutral forms are always more stable than the corresponding zwitterionic ones. The energy difference between the neutral form and the corresponding zwitterionic one is around 10 kJ/mol. This result agrees with that by Melo *et al.*[3]

In Fig. 3 are shown the energy changes involved in the inclusion process of α-CD with the salt bridge in the NT(H), and the complexes structures corresponding to the lowest energy in the curve. As seen, the optimized complex indeed reflects substantial inclusion of the guest in the central cavity of α-CD. Similarly, the optimized structures of the α-CD complexes with the salt bridge in the NT(CH_3), NT(H), ZT(H) and ZT(CH_3) forms were also obtained and shown in Fig. 4.

The energies of the α-CD complexes are especially interesting. As seen from Table 3, it is clear that the α-CD complex with the salt bridge in the ZT(H) or ZT(CH_3) form has a lower energy than the corresponding one in the NT(H) or NT(CH_3) form. As the energy difference between the zwitterionic form and the corresponding neutral one is around 30 kJ/mol, it can be concluded that a hydrophobic environment provided by the internal cavity of α-CD should stabilize the zwitterionic salt bridges.

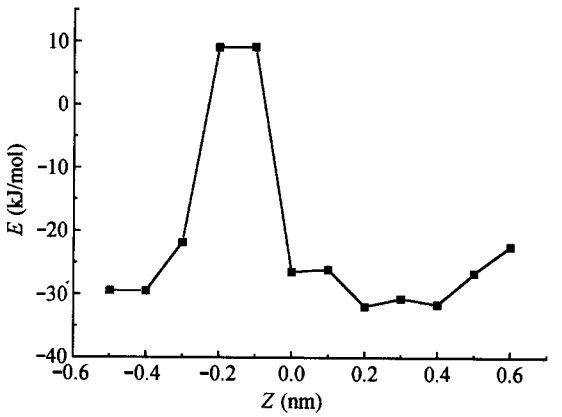

Fig. 3 Progression of the energy when simulating the inclusion complexation of the salt-bridge into α-CD cavity.

The above behavior is understandable if we notice that 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). As known, cyclodextrins have significantly large dipole moments[11] and the dipole-dipole interaction is an important driving force in CD complexation.[12] Therefore, the complexation of α-CD with the zwitterionic salt bridge is more favorable in energy than that with the neutral one in term of electrostatic interaction. In addition, it is clear that in the complexes hydrogen bonds are formed between the salt bridge and the α-CD hydroxyls, which might also help to stabilize the zwitterionic salt bridge. In fact, similar groups such as OH and SH are also present in real proteins and their interactions with the salt bridges have been considered to be a factor contributing to the stability of ion pairs.[5]

In addition, the structure parameters of the arginine-carboxylate salt bridge bound by the α-CD cavity are listed in Table 4. Comparing the values with those in vacuum, it can be seen that α-CD complexation does, but not to a significant extent, affect the structure of the salt bridge. Therefore, the change in the stability of the different forms of the salt bridge is caused mainly by a noncovalent species-environment interaction.

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

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

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

Form	α (deg.)	Distance (nm)						
		C(1)—C(5)	O(8)—H(7)	H(7)—N(6)	O(2)—H(3)	H(3)—N(4)	O(8)—N(6)	O(2)—N(4)
ZT(H)	174.1	0.384	0.154	0.108	0.150	0.110	0.262	0.258
NT(H)	172.1	0.400	0.189	0.102	0.104	0.161	0.290	0.264
ZT(CH_3)	58.8	0.383	0.145	0.111	0.156	0.108	0.256	0.263
NT(CH_3)	54.3	0.400	0.103	0.162	0.189	0.102	0.265	0.290

Table 3 Total energies of four forms of the α-CD-salt-bridge complexes

Form	α-CD-ZT(H)	α-CD-NT(H)	α-CD-ZT(CH_3)	α-CD-NT(CH_3)
E (kJ/mol)	-10857643.88	-10857605.35	-10857613.94	-10857603.96

Table 4 Structure parameters of four forms of the methylguanidinium-acetate pair bound in α-CD

Form	α (deg.)	Distance (nm)						
		C(1)—C(5)	O(8)—H(7)	H(7)—N(6)	O(2)—H(3)	H(3)—N(4)	O(8)—N(6)	O(2)—N(4)
α-CD-ZT(H)	160.41	0.395	0.174	0.102	0.171	0.105	0.270	0.275
α-CD-NT(H)	166.61	0.413	0.182	0.101	0.098	0.177	0.283	0.275
α-CD-ZT(CH_3)	60.81	0.391	0.171	0.103	0.174	0.103	0.273	0.267
α-CD-NT(CH_3)	55.88	0.411	0.098	0.177	0.182	0.101	0.274	0.282

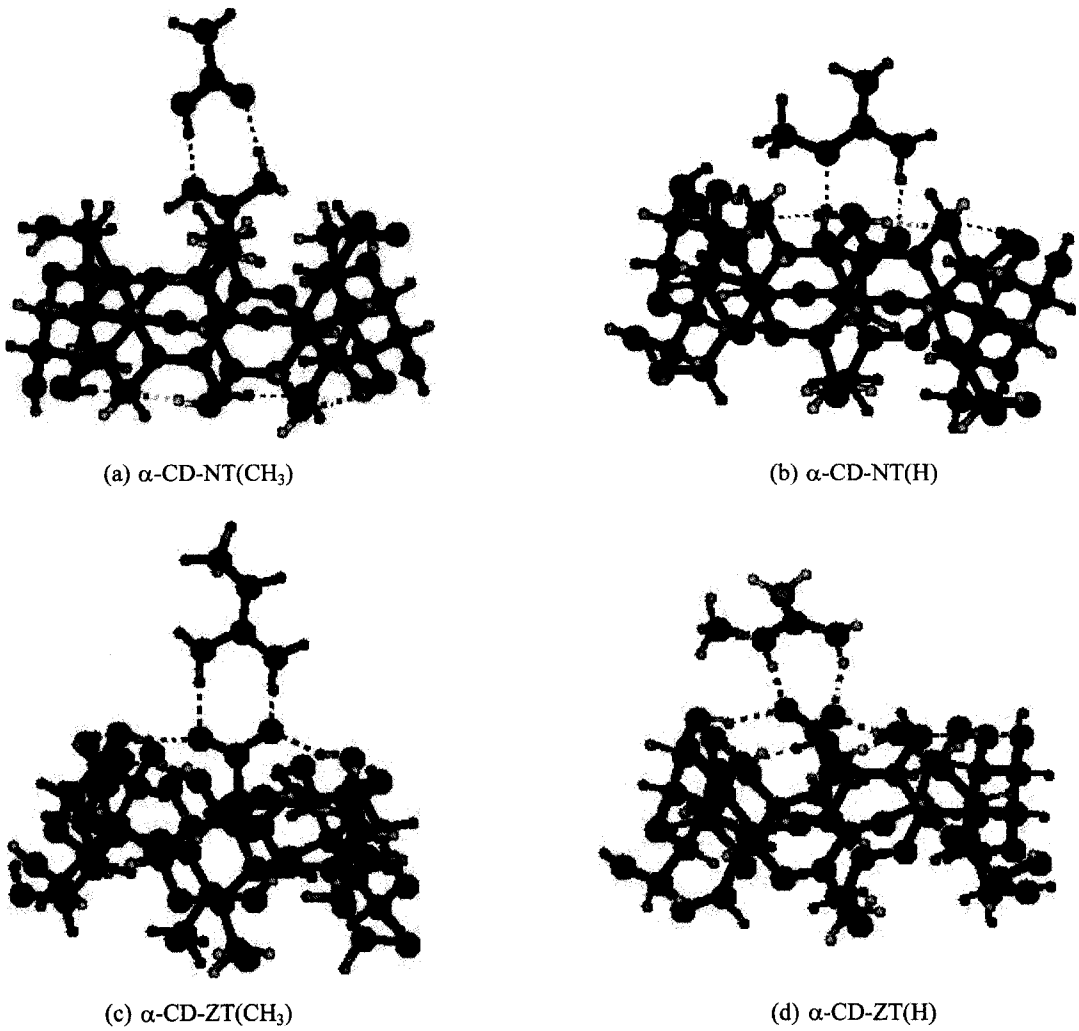

Fig. 4 Structure of four forms of complexes optimized by PM3 method.

Conclusion

The zwitterionic and neutral forms of arginine-carboxylate salt bridge were studied by B3LYP/6-311G(d, p)//PM3 method. It was found that in gas phase, the neutral forms were more stable than the zwitterionic counterparts. However, when bound by α-cyclodextrin, the zwitterionic forms became more stable than the corresponding neutral ones. It was suggested that the hydrophobic environment provided by the cyclodextrin cavity led to such behavior. Therefore, the salt bridge still could be in a zwitterionic form in the hydrophobic interior of the real proteins.

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