The Influence of Dielectric Constant on Ionic and Non-polar Interactions

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This work is focused on analyzing ion-pair interactions and showing the effect of solvent induced inter-atomic attractions in various dielectric environments. To estimate the stability of ion-pairs, SCI-PCM *ab initio* MO calculations were carried out. We show that the solvent-induced attraction or 'cavitation' energy of the ion-pair interactions in solution that arises mainly from the stabilization of the water molecules by the generation of an electrostatic field. In fact, even the strong electrostatic interaction characteristic of ion-pair interactions in the gas phase cannot overcome the destabilization or reorganization of the water molecules around solute cavities that arise from cancellation of the electrostatic field. The solvent environment, possibly supplemented by some specific solvent molecules, may help place the solute molecule in a cavity whose surroundings are characterized by an infinite polarizable dielectric medium. This behavior suggests that hydrophobic residues at a protein surface could easily contact the side chains of other nearby residues through the solvent environment, instead of by direct intra-molecular interactions.

Key Words : Ionic interections, *ab initio*, SCI-PCM, Solvent induced attraction

Introduction

Electrostatic interactions play an important role in determining the structure and function of biological molecules.[1-3] Charged groups located at the exterior region of proteins are crucial for expressing their surface properties. Although pairing of charged groups observed in proteins is usually between opposite charges, pairings of like-charged groups have been found in crystal structures.[4-8] According to continuum solvation calculations, some like-charged organic ion-pairs become attractive upon hydration.[9,10] Repulsive interactions seen in the gas phase between like-charged ion pairs can be reduced in aqueous solution and become attractive, for example, in the guanidinium ion pair.[13,14] Also, attractive interactions between oppositely charged ion pairs decrease upon hydration, and become repulsive, for the methyl ammonium-methyl acetate ion pair. Several computational studies have been performed to illuminate the behavior of such ion pairs in aqueous solution.[11-13]

Since interactions involving ionic groups are very important for biological activity, it is necessary to understand and describe the stability of ion- pair interactions in biological macromolecules in relation to their environment. Tidor *et al.* suggested that the strength of salt bridges depends on the choice of the internal protein dielectric constant and ionic strength used in continuum electrostatic models.[15] According to the results, all the salt bridges they studied were electrostatically destabilizing by a substantial amount, ~2.5-6.0 kcal/mol.

Hydrophobic interactions involving molecules with ion-pairs must include solvent induced attraction between the non-polar groups in aqueous solution. The hydrophobic effect is considered to be an important driving force in a large variety of molecular recognition processes and the folding of globular proteins, the assembly of micelles and lipid bilayer membranes. Hydrophobicity is one of the most conserved characteristics of both buried and exposed amino acids during mutagenesis.[16] Pratt and Pohorille showed the importance of the amounts of water molecule contributing to solvent induced attraction by examining simulations of *n*-dodecane in different solvents.[17] The origin of hydrophobicity is the destabilization of water by cavity formation. According to Monte Carlo and molecular dynamics simulations, hydrophobic attraction is mainly the result of a favorable free energy change of water by reducing the solute-exposed cavity as two molecules approach.[18-21] Vila *et al.* showed that the stabilization of a lysine ion pair is due to a combination of hydrophobic interactions and solvent polarization effects.[22] Thus, the structure of a protein in water is primarily a consequence of two interactions, ion-ion and hydrophobic.

In this work, to understand the solvent effect, the stabilities of some ion pairs in various dielectric environments - both ionic polar and non-polar interactions - are determined with SCI-PCM[23] MO calculation. For the ionic polar interactions, both like and unlike charged ion pairs are used as *charge-charge interaction* models, positive-positive and positive-negative ionic pairs, on the protein surface. The non-polar interactions represent *solvent induced attraction* between the

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hydrophobic non-polar residues on the protein surface.

Calculations

The interaction between the ionizable side chains of such amino acid residues as Asp, Lys and Arg is modeled by the methyl acetate, methyl ammonium, and guanidinium moieties. We divide these models into two parts, where one part is the ion-pair facing each other, such as $CH_3CO_2^-\cdots{}^+NH_3CH_3$, $CH_3NH_3^+\cdots{}^\ddagger NH_3CH_3$, $C(NH_2)_3^+\cdots{}^\ddagger(NH_2)_3C$, and the other part is the one member of the ion pair facing a hydrocarbon groups, such as ${}^+NH_3CH_3\cdots CH_3NH_3^+$, ${}^+NH_3$-$CH_2CH_3\cdots CH_3CH_2NH_3^+$, and ${}^+NH_3(CH_3)_3C\cdots C(CH_3)_3NH_3^+$.

All monomeric geometries are optimized with the HF 6-31+G* basis set in the gas phase and with various dielectric media. To determine the stability of each ion-pair using continuum (ε = 4, 20, 40, 60, 78.3) solvation, the sinlge point energy calculations for the intermolecular binding energy calculations are carried out with SCI-PCM[23] implemented in Gaussian 94.[24] An isodensity level of the electron distribution 0.0004 a.u. is employed. According to No, *et al*.,[9] the binding energy of the complex in the dielectric continuum is estimated as the difference between the total energy of the complex and the sum of the total energies of the monomers.

The solvation energy, $E_{solvation}$ (R_A:X) of ion pairs can be simply decomposed as,[25]

$$E_{solvation}(R_A{:}X) = E_A(R_A) + E_{A\cdots X}(R_A) + E_X(R_A) \quad (1)$$

where A is the solute and X is the solvent, R_A represents the conformation of the solute A. E_A, $E_{A\cdots X}$ and E_X represent the energy of the solute, the interaction energy between solute and solvent, and the energy of the solvent, respectively.

For describing the two molecules with various dielectric constants, the stabilization energy of the solute pair A and B in the solvent X, $E_{stabilization}$ (R_A, R_B:X), can be written,

$$\begin{aligned} E_{stabilization}(R_A, R_B{:}X) &= \{E_{AB}(R_A, R_B) - (E_A(R_A) + E_B(R_B))\} \\ &+ \{E_{AB\cdots X}(R_A, R_B{:}X) - E_{A\cdots X}(R_A{:}X) \\ &+ E_{B\cdots X}(R_B{:}X))\} + \{E_X(R_A, R_B) - (E_X(R_A) + E_X(R_B))\} \end{aligned} \quad (2)$$

Here $E_{AB}(R_A, R_B)$, $E_A(R_A)$, and $E_{AB\cdots X}(R_A, R_B{:}X)$ represent the energy of the $A\cdots B$ pair at (R_A, R_B), the energy of A at R_A, and the interaction energy of the $A\cdots B$ at (R_A, R_B) with solvent X, respectively. $E_X(R_A, R_B)$ and $E_X(R_A)$ represent the energy change of the solvent by the solute $A\cdots B$ and solute A, respectively.

With the continuum solvation model, the stabilization energy is expressed approximately as

$$\begin{aligned} E_{stabilization}(R_A, R_B{:}X) &= \Delta E_{solute}(R_A, R_B) - \Delta E_{solute-solvent} \\ &(R_A, R_B, X) + \Delta E_{solvent}(R_A, R_B, X) \end{aligned} \quad (3)$$

The first and second terms of the right hand side of the equation can be calculated using *ab initio* SCI-PCM MO and equation (3) then becomes

$$\begin{aligned} E_{stabilization}(R_A, R_B{:}X) &= [E_{SCIPCM}(R_A, R_B, \vec{\varepsilon}(X)) \\ &- \{E_{SCIPCM}(R_A, \vec{\varepsilon}(X)) + E_{SCIPCM}(R_B, \vec{\varepsilon}(X))\}] \\ &+ \Delta E_{solvent}(R_A, R_B, X) \end{aligned} \quad (4)$$

where $\vec{\varepsilon}(X)$ is the dielectric constant of the solvent, X. The main contribution is from the difference in cavitation energy, $\Delta E_{solvent}$ (R_A, R_B, X). In this work, $\Delta E_{solvent}$ (R_A, R_B, X) is approximated as the cavitation energy difference, $\Delta\Delta E_{cav}$ (R_A, R_B, X), because the polarized restructuring energy cannot be calculated in the continuum model.

$$\begin{aligned} &\Delta\Delta G_{cav}(R_A, R_B, X) = \\ &\Delta G_{cav}(R_A, R_B, X) - \{\Delta G_{cav}(R_A, X) + \Delta G_{cav}(R_B, X)\} \end{aligned} \quad (5)$$

where cavitation energy of monmer A, ΔG_{cav} (R_B, X), can be expressed,[26]

$$\Delta G_{cav}(R_B, X) = C + \sum_k \gamma_X A_k(R_A) \quad (6)$$

where γ_k is the surface tension of the solvent $A_k(R)$ is the solvent accessible surface area of kth atom.

The cavitation energy difference, $\Delta\Delta G_{cav}$ (R_A, R_B, X), becomes

$$\begin{aligned} &\Delta\Delta G_{cav}(R_A, R_B, X) = \\ &\gamma_X \sum_k \{A_k(R_A, R_B, X) - (A_k(R_A, X) + A_k(R_B, X))\} \end{aligned} \quad (7)$$

where $A_k(R_A, R_B, X)$, $A_k(R_A, X)$ and $A_k(R_B, X)$ are the solvent accessible surface area of the kth atom in AB complex, A molecule, and B molecule, respectively.

Therefore, the stabilization energy is obtained with the following formula.

$$\begin{aligned} &E_{stabilization}(R_A, R_B, X) = \\ &\Delta E_{SCIPCM}(R_A, R_B, \vec{\varepsilon}(X)) + \Delta\Delta G_{cav}(R_A, R_B, X) \end{aligned} \quad (8)$$

The $\Delta\Delta G_{cav}$ is obtained only for aqueous solution because the other dielectric constants are not applied.

Results and Discussion

The formulas described above allow us to directly determine the relative effect of increasing the strength of binding energy for various dielectric constants. One part of the calculation is concerned with the ionic electrostatic interaction for closely facing charges and the other part is related to the solvent induced attractions for ion-pairs that are facing carbon groups. Table 1 tabulated the stabilization energies of each ion pairs with different dielectric constants.

Charge-Charge Interaction. According to Figure 1, the like-charged ionic molecules complexes have no energy minimum in the gas phase and have larger minimum binding energy as a function of increasing dielectric constant. In contrast to this, $CH_3CO_2^-\cdots{}^\ddagger NH_3CH_3$ ion pair shows decreasing binding energy.

These models, $CH_3CO_2^-\cdots{}^\ddagger NH_3CH_3$, $C(NH_2)_3^+\cdots{}^\ddagger(NH_2)_3C$ and $CH_3NH_3^+\cdots{}^\ddagger NH_3CH_3$ ion pairs, show that the stabilization energy primarily is influenced by different dielectric environment as well as the native intramolecular character. In the case of $CH_3CO_2^-\cdots{}^\ddagger NH_3CH_3$, the strong electrostatic interaction in the gas phase results in gradual destabilization into polar aqueous solvent for all cases studied. Also, the stabilization energies of $C(NH_2)_3^+\cdots{}^\ddagger(NH_2)_3C$ ion pairs are

Table 1. The calculated stabilization energy and the minimum distance between C_a-carbons at various dielectric constants

	$CH_3CO_2^-\cdots{}^+NH_3CH_3$	$C(NH_2)_3^+\cdots{}^+(NH_2)_3C$	$^+NH_3CH_3\cdots CH_3NH_3^+$	$^+NH_3CH_2CH_3\cdots CH_3CH_2NH_3^+$	$^+NH_3(CH_3)_3C\cdots C(CH_3)_3NH_3^+$
Gas	-115.58 (r = 3.0 Å)	–	–	–	–
ε = 4.0	-33.14 (r = 3.0 Å)	–	–	–	–
ε = 20.0	-1.54 (r = 3.0 Å)	0.22 (r = 3.0 Å)	0.48 (r = 2.5 Å)	-0.87 (r = 4.5 Å)	–
ε = 40.0	-8.09 (r = 3.0 Å)	-1.63 (r = 2.5 Å)	-0.74 (r = 2.2 Å)	-2.49 (r = 4.5 Å)	0.62 (r = 5.5 Å)
ε = 60.0	-7.15 (r = 3.0 Å)	-2.29 (r = 2.5 Å)	-1.51 (r = 2.3 Å)	-2.94 (r = 4.5 Å)	-1.55 (r = 5.0 Å)
ε = 78.3	-6.77 -8.42[a] (r = 3.0 Å)	-2.68 -5.13[a] (r = 2.5 Å)	-1.64 -2.75[a] (r = 2.2 Å)	-3.15 -5.30[a] (r = 4.0 Å)	-0.99 -2.77[a] (r = 5.5 Å)

[a]Cavitations corrected stabilization energy by Eq. (8).

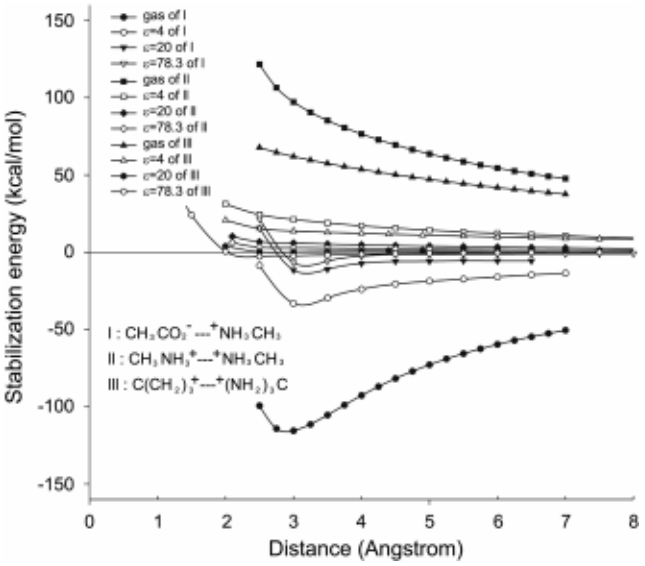

Figure 1. The stabilization energies of each ion pair in different dielectric medium are plotted against the inter-ionic distances for ion pairs facing the charged groups with each other.

obtained -1.63, -2.29 and -5.13 kcal/mol at the minimum intermolecular C⋯C distance of 2.5 Å with $\varepsilon = 40.0$, 60.0 and 78.3 respectively, even though they have the electrostatic repulsion in gas phase (Table 1). The agreement here is good, since this interaction is known to be attractive with a deep and large minimum at a C⋯C distance of 3.3 Å by Boudon *et al*'s system.[13] This ion-pair has both repulsion from the retention of like charges and attraction that arises from the steric and bulky shape. With increasing the dielectric constant, the strong repulsion between the like charges diminishes because of intervention of some implicit water molecules. The oppositely charged ion pairs model, $CH_3NH_3^+\cdots{}^+NH_3CH_3$, with the cavitation correction has the unstable stabilization energy, $E_{stabilization}(R_A, R_B, X)$, 1.59 kcal/mol at 2.5Å in aqueous solution, respectively.

As the results show, the cavitation energies include both the size and the shape of the solvent as well as solute molecules. The solvent properties are described in terms of the definite dielectric constant and the surface tension. With the expended cavitation energy in Eq. (8), the stabilization energies for the charge-charge interaction gradually increases, as shown in Figure 3. The stabilization energy added to the cavitation energy provides a physically more complete treatment of solvation phenomena.

Solvent Induced Attraction. In the system, the methyl group facing each other in the different dielectric constant shows that the relative stabilization of the ionic molecules explains their environment containing the solvent molecule and being in the space occupied by the solvent molecule, like water, as well as their intrinsic interaction.

We have also studied the $^+NH_3CH_3\cdots CH_3NH_3^+$, $^+NH_3CH_2CH_3\cdots CH_3CH_2NH_3^+$, and $^+NH_3(CH_3)_3C\cdots C(CH_3)_3NH_3^+$, ion pairs, where the carbon atoms are facing each other. The results show the important and meaningful aspect of the solvent induced attraction due to the hydrophobic as well as electrostatic interactions. The stabilization energies of the ionic pairs result in increased stability as a function of increasing dielectric constant as for the behavior of the previous models. Above all, we note that the stabilization energies increase according to the carbon size without confronting charges (Figure 2). Also it shows that the stabilization energy adds to the cavitation energy of each ion-pair at $\varepsilon = 78.3$ as seen in Figure 3. The cavitation energy is entirely due to solvent reorganization around the cavity, and is proportional to the accessible surface area and surface tension coefficient, γ, in Eq. (6), derived from the solubility properties of nonpolar hydrocarbons. These observations might suggest more increased stability for the $^+NH_3CH_2CH_3\cdots CH_3CH_2NH_3^+$ ion-pair than for the $^+NH_3(CH_3)_3C\cdots C(CH_3)_3NH_3^+$ ion-pair, as a result of this additional cavitation energy term.

In particular, $^+NH_3CH_2CH_3\cdots CH_3CH_2NH_3^+$ ion pair such as -5.30 kcal/mol at 4.0 Å, has larger minimum of stabilization energy than $^+NH_3(CH_3)_3C\cdots C(CH_3)_3NH_3^+$, with -2.77 kcal/mol at 5.5 Å, in aqueous solution as shown in Table 1. In $^+NH_3CH_2CH_3\cdots CH_3CH_2NH_3^+$ ion pair, the charge groups of them result in stabilizing the water molecules due to their strong electrostatic field and the other part, ethyl groups

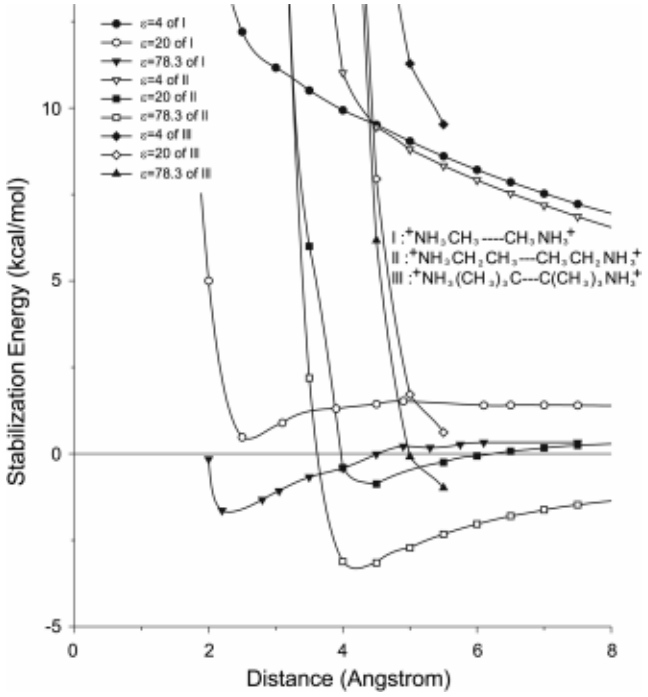

Figure 2. The stabilization energies of each ion pair in different dielectric medium are plotted against the inter-ionic distances for ion pairs facing the hydrocarbon groups with each other.

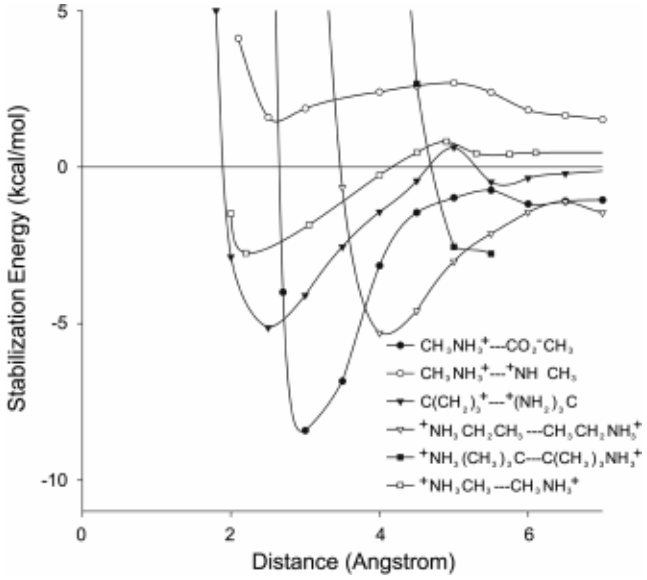

Figure 3. The stabilization energy adding in the cavitation energy of each ion pair at ε = 78.3.

confronting with each other, has their induced hydrophobic attraction, called the solvent induced attractions. Thus the ion pair with water molecules becomes stable unlike the other ion pair models. On the other hand, the $^{+}NH_3(CH_3)_3C\cdots C(CH_3)_3NH_3^{+}$ ion pair may relatively loosen the interaction between the charged group and water molecule and become less stable than the $^{+}NH_3CH_2CH_3\cdots CH_3CH_2NH_3^{+}$ ion pair as possessing more space instead of water molecules. *t*-butyl groups, facing each other, drive to maximize a hydrophobic interaction with their bulky size. Also, the methyl groups of the $^{+}NH_3CH_3\cdots CH_3NH_3^{+}$ ion pair have less hydrophobic interaction although the other charged parts stabilize water molecules surrounding them more. Also the results for *t*-butyl ammonium ion pairs show some minimum stabilization energy but only for -1.55 and -2.77 kcal/mol at ε = 60.0 and 78.3. Namely, it means that the weak interaction is maintained in an aqueous solution. From Figure 2, near about 6.0 Å, they may show stronger repulsion in contrast with other hydrophobic ion pairs due to the convergence problem.

The results describing the effect of the solvent allow us to stress the influence of the environment in the binding process and the stability between those ionic pair complexes. A hydrophobic surface region can be defined as a continuous piece of surface, which is formed exclusively by nonpolar atoms and is not occupied by water molecules bound to polar atoms.[27] As an example, we consider the x-ray crystallographic structure of staphylococcal nuclease, containing two interacting pairs of Lys residue. The aliphatic portions of the lysine side chains form strong hydrophobic contacts bringing the N_ζ atoms of neighboring side chain at distances of 4.1 and 6.2 Å for the K63-K64 and K70-K71 pairs in the structure of staphylococcal nuclease.[28] In agreement of our results, x-ray crystallographic structure of staphylococcal nuclease[28] and Vilar *et al.* performed electrostatically driven Monte Carlo (EDMC) simulation for the sequence Ac-$(Lys)_6$-NMe using the ECEPP/3 force field.[22] The ionizable side chain of Lys amino acid residue, the $^{+}NH_3CH_2CH_3\cdots CH_3CH_2NH_3^{+}$ ion pair, was modeled by SCI-PCM calculations. The intermolecular distance within 4.0 Å that had the minimum stabilization energy of $^{+}NH_3CH_2CH_3\cdots CH_3CH_2NH_3^{+}$ ion pair, respectively, was similar to interactions among Lys side chains positioning the NH_3^{+} groups of x-ray crystallographic structure of staphylococcal nuclease at 4.1 Å[28] and EDMC simulations at the range 5.3-7.0 Å.[22]

Through our calculation, we can determine the existence of the minimum point depending on the size of ion pair and their steric effect. This would suggest that the hydrophobic residues in the protein surface easily contact the side chains of other residues as a result of the solvent environment instead of intra-molecular interaction.

Conclusion

The results presented here suggest that charge-charge energy effects involving oppositely charged ion-pairs as well as non-polar groups, depend on the interactive effects between each ion pairs and water for their solvation as well as the intra-interaction between their ion pairs. This environment implements that the solute molecule, possibly supplemented by some specific solvent molecules, may be placed in a cavity surrounded by an infinite polarizable dielectric constant. We would like to implicate that the interaction of ionic molecules within the different dielectric constants is more reasonable with the unpredictable protein interior environment. This analysis allows us to directly

determine the relative effect of increasing the cavity polarity on the strength of binding energy with the different dielectric constants.

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