Charge-Transfer Interactions in Macromolecular Systems: A New View of the Protein/Water Interface

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In this communication we employ linear-scaling quantum mechanical methodologies to carry out the first fully quantum mechanical calculation on a protein/water system (∼4300 atoms total). These calculations demonstrate for the first time that the superposition of a number of small charge transfer (CT) interactions at the protein/water interface results in a substantial transfer of charge from the protein surface to the surrounding solvent. Furthermore, we show that the charge-transfer interaction is a significant contributor to the overall interaction energy in hydrogen bonding complexes—even more so than the closely related polarization interaction. At the end of this note we discuss the theoretical and experimental ramifications of the charge-transfer interaction for biomolecules in aqueous solution.

A charge-transfer interaction[1] has two important effects on the complexation process: (1) it stabilizes (or destabilizes) complex formation—this affects the total interaction energy—and (2) it results in the net partial transfer of charge (i.e., electrons) from one complexing molecule to the other. Little work has focused on this interaction in protein systems (as opposed to electrostatic, exchange-repulsion, and polarization[2,3]), which is surprising because it is known that the magnitude of the CT interaction energy is in many cases twice that of the polarization energy.[1,4] Moreover, it also has been generally assumed that the net result of the CT interaction (i.e., a net transfer of charge) in most cases is very small (a few hundredths of an electron) and that its overall effect on the charge distribution of a molecule is, therefore, negligible. This, indeed, turns out to be the case for two small molecules interacting with one another (e.g., the water dimer).

The major cold shock protein of *E. coli*, Cold-Shock protein A (CspA), a small hydrophilic protein with 69 amino acid residues, was used as a model system in our study. We chose to carry out calculations on this system due to its relatively small size and its near neutral charge under physiological conditions. The crystal structure at 2.45 Å resolution was used as the initial model of CspA.[5] Since under normal physiological conditions proteins exhibit rich conformational dynamics which play an essential role in protein function,[6,7] we decided to study the dynamics of CspA by performing molecular dynamics simulations

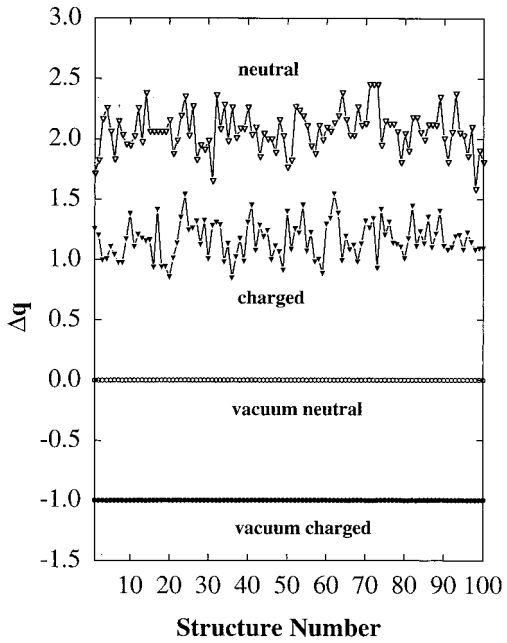

Figure 1. Fluctuations in the total charge of CspA in vacuum and in aqueous solution.

on the CspA water system using the AMBER[8] force field with the TIP3P[9] water model and the SANDER[10] molecular dynamics (MD) module. From a 500 ps sampling phase we extracted 100 coordinate sets that we then carried out single point semiempirical (PM3[11]) self-consistent field (SCF) calculations on using the DivCon program.[12] Each single point PM3 calculation required ∼4 h of computer time on a SGI Origin 200 workstation. Thus, these are still expensive calculations, but in the absence of our linear-scaling quantum mechanical code[12] these calculations would have been impossible to carry out in a reasonable amount of time. We studied CspA in two charged states, one in which the system has a unit negative charge and the other in which it has a net neutral charge.[13] An in vacuo simulation of CspA was also performed to serve as a control. Figure 1 depicts the total charge on the protein over the period of the simulation. The charges presented are Coulson charges[14] and are not electrostatic potential fit[15] charges. ESP charges are better at reproducing the multipolar (e.g., dipole, quadrupole, etc.) characteristics of a molecule than are Coulson charges, but it is impossible to use the ESP fitting procedure on systems as large as those studied herein.[15] As expected, the net charge in vacuo was integral in all cases (i.e., −1 or 0). When we calculated the protein charge in the presence of the water molecules contained in the system we found that there was a significant amount of charge transferred from the protein surface to the surrounding solvent. The average total charge on the protein was 1.167 with a 13% fluctuation for the

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Table 1. The Average Charge ($\langle\Delta q\rangle$) Transferred to Water by Charged/Polar Residues of CspA

residue	$\langle\Delta q\rangle$	residue	$\langle\Delta q\rangle$	residue	$\langle\Delta q\rangle$
Ser 1	+0.35	Asp 24	−0.19	Asp 45	−0.21
Lys 3	+0.05	Lys 27	+0.07	Glu 46	−0.22
Lys 9	+0.07	Asp 28	−0.21	Glu 55	−0.22
Asp 14	−0.21	Asp 39	−0.18	Lys 59	+0.06
Lys 15	+0.05	Lys 42	+0.06	Leu 69	−0.47
Asp 23	−0.22				

[a] A "+" sign indicates that the residue is accepting charge (*i.e.*, electrons) from its surroundings, while a "−" sign indicates that this residue is donating charge (*i.e.*, electrons) to its surroundings.

negatively charged CspA system, and 2.07 with an 8% fluctuation for the neutral case. Thus, while the net charge of the system consisting of the protein and water was conserved, we found that the protein transfers roughly two (2) units of charge to the solvent regardless of the initial charge state of the protein.

To determine if specific groups of atoms or if all atoms contribute equally to the observed charge transfer we analyzed the contribution of each amino acid residue to the overall CT. We were able to track down several residues that were the main contributors to the observed transfer of charge (see Table 1). We found, albeit not surprisingly, that the atoms involved are the polar and charged residues, Lys, Asp, and Glu. Thus, for example the Lys residues each accept ∼0.05 e of charge, while the Glu/Asp residues each transfer about 0.2 e of charge to the surrounding solvent environment. Ser 1 is on the N-terminus of CspA and is positively charged so it accepts a large (0.35 e) amount of charge, while Leu 69, which is the C-terminus, is negatively charged and donates a large amount of charge (0.47 e) to the surrounding environment. The remaining residues within CspA transfer or accept ∼0.05 e or less. Thus, in terms of the transfer of charge the Asp/Glu and the C-terminal protein are the most important groups overall (*i.e.*, the carboxylate group), while positively charged groups such as Lys tend to slightly counterbalance the net transfer of charge. We note that CspA does not have an Arg residue so we are unable to comment on how much charge it might transfer to solvent.

The observation that charge is transferred between the protein/water interface is interesting, but it does not address what is the magnitude of the charge-transfer interaction energy. To estimate the strength of this interaction we have made use of the Morokuma decomposition method[1] as implemented by GAMESS,[16] and the results of these calculations on a series of hydrogen bonded complexes are presented in Table 2. These systems were chosen since they are representative of the types of interactions present at the protein/water interface. The calculations could only be carried out at the 6-31G level of theory due to convergence problems at higher levels of theory. Thus, while the absolute energies may be too high, we expect that the relative energy ordering for the various interaction energies will not change significantly. Furthermore, it has been shown that the Morokuma methodology can be unstable when very large basis sets (i.e., much larger than the 6-31G basis set) are used in calculations of this type.[4] The electrostatic portion of the interaction in all cases is the leading contributor to formation of a stable hydrogen bonded complex. The exchange interaction is destabilizing, while the polarization interaction is ∼10% of the total interaction energy in all cases as has been suggested previously.[17] The CT interaction accounts for ∼20% of the total interaction energy in all cases. Thus, the CT interaction is more important than the polarization interaction by about a factor of 2.

Table 2. Decomposition of the Total Interaction Energy (ΔE_{tot}) into Polarization (ΔE_{pol}), Charge Transfer (ΔE_{CT}), Exchange Repulsion (ΔE_{ex}), and Electrostatic (ΔE_{el}) Contributions[a]

system	ΔE_{pol}	ΔE_{CT}	ΔE_{ex}	ΔE_{el}	ΔE_{totals}
methylformamide−water	−1.36	−2.84	7.40	−14.11	−11.45
acetate anion−water	−2.53	−5.18	16.34	−32.53	−25.61
methylamine−water	−0.46	−0.76	2.29	−3.81	−2.85
methylammonium−water	−3.50	−4.71	14.72	−31.26	−24.63
water−water	−0.80	−1.70	6.31	−11.13	−7.49

[a] All energies in kcal/mol.

In summary, we have carried out the first fully quantum mechanical calculations on an explicit protein/water system (∼1000 protein and 3300 water atoms total) using the semiempirical parametric model 3 (PM3) Hamiltonian. From the calculations presented herein we have arrived at two significant conclusions: (1) We have found that charge-transfer interactions account for ∼20% of the total interaction energy involved in hydrogen bonded complexes and this is twice the effect polarization has on calculated interaction energies. While the latter observation supports previous conclusions[1,4] we more importantly (2) observe that CT interactions result in the net transfer of charge from the surface of a protein to the surrounding solvent and that this transfer of charge can be quite substantial. Given these observations we conclude that CT interactions as well as the transfer of charge could have a significant impact on both our experimental and theoretical understanding of biomolecules in aqueous solution.

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