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Effects of Hydration on the Electronic Structure of an Enzyme: Implications for the Catalytic Function

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Water constitutes 70−90% of the mass in most living systems, and it plays an important role in maintaining structure and function of proteins. The so-called hydrophobic interaction arises from hydration of nonpolar molecules, leading to a marginal stability of the native structure of a protein relative to the denatured state.[1] In addition to such a conventional picture, new aspects of the role of hydration in protein chemistry have been revealed by recent quantum chemical studies.[2] For example, a significant amount of charge transfer was found to occur between a protein and the surrounding water molecules. This mainly changes the electronic structure of the protein−water interface and may affect the stability of the protein−substrate complex.[2b] Development of linear-scaling molecular orbital methods[3] has extended the realm of quantum chemical calculation to elucidation of the electronic structure of hydrated proteins. Unlike charge transfer, the polarization of a solvent is expected to exert its influence on almost all residues because of its long-range nature. Here, we study how it influences the active orbitals of an enzyme, ribonuclease T_1 (RNase T_1). It will be shown that hydration causes a large modification to the energy and spatial distribution of the molecular orbitals of the protein. Interestingly, the frontier orbitals[4] of the hydrated protein are found to be localized on several functionally important residues.

The linear-scaling molecular orbital method used here is MOZYME,[5] which is implemented into the MOPAC2000 program.[6] The initial coordinate of the protein studied was taken from the Protein Data Bank (the PDB entry code is 4GSP[7]). Hydrogen atoms were added using the Insight II program. The positions of all the hydrogen atoms and the heavy atoms of all the side chains were optimized by the MOZYME calculation. All of the other geometries concerning the heavy atoms of the backbone were fixed to the experimental values. The cutoff distance was chosen to be 12 Å. Below that distance, the interaction between two atoms is represented by exact NDDO approximation, where the AM1 Hamiltonian[8] was used. The solvent-effect calculation was carried out using a continuum model known as COSMO.[9] Then, the dielectric constant of solvent (water) was taken to be 78.

As expected, the orbital energies of the protein exhibited a band-like structure (data not shown). All of the orbital energies were found to be drastically lowered by hydration. The average orbital energy over all of the occupied orbitals was −19.91 eV for the solution state, which was lower than that for the gas phase by 7.36 eV. To examine the detail about the effect of hydration, we picked up only the lone pairs of the backbone nitrogen atoms, because these orbitals are distributed over the entire molecule. Figure 1, a and b, shows the energy diagrams in the gas phase and in water, respectively. As a result of hydration, the orbital energies are lowered, and the density of states per unit energy tends to become high. In general, the interior of the protein should be highly anisotropic but have some regularity determined by its tertiary structure. Thus, the lone pairs of the backbone nitrogen atoms are located at environments differing in electrostatic potentials. This brings about a significant amount of diversity in orbital energies in the gas phase (Figure 1a).[10] However, the hydration dramatically decreases such an anisotropy.

To elucidate the physical origins of the hydration-induced orbital changes, we carried out two analyses. Figure 1c shows the energy diagram for the protein in which all of the ionizable residues located on the water-accessible surface are neutralized. The average orbital energy in Figure 1c was −19.09 eV, very close to that in Figure 1b. Thus, the major origin of the orbital energy changes is ascribed to the shielding of the electric fields from the ionized residues on the surface due to the dielectric effect of water. Next, we investigated how the delocalized nature of the molecular orbitals is influenced by hydration. Here we define "delocalization parameter D" as follows:[11]

$$\phi = \sum_{i=1}^{N} c_i \chi_i$$

$$\sum_{i=1}^{N} c_i^2 = 1$$

$$D = 1/\sum_{i=1}^{N} c_i^4$$

where ϕ is a molecular orbital, χ_i and c_i are the ith atomic orbital and its coefficient, respectively, and N is the total number of atomic orbitals, the value of which is 3919 for RNase T_1. If the coefficients c_i's are equally populated over the N atomic orbitals, the value of the delocalization parameter D should equal N. Otherwise, the value is smaller than N. Figure 2 shows the plot of D against orbital energy. On the whole the D values for the solution state are larger than that for the gas state. D/N may be a more general measure for delocalization.[12] The D/N value averaged over all of the occupied orbitals was evaluated to be 0.019 and 0.017 for the solution and gas states, respectively. Therefore, the hydration causes about 10% increase in the degree of delocalization of the molecular orbitals relative to that in the gas phase, consequently contributing to the lowering of the orbital energies.

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(4) In a strict sense, the frontier orbitals mean the highest-occupied molecular orbital (HOMO) and the lowest-unoccupied molecular orbital (LUMO) in a closed shell molecule. However, in this study its meaning is extended to cases including several orbitals energetically close to HOMO and LUMO.
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(10) We confirmed that there is good linearity between the orbital energies of the lone pairs and the electrostatic potentials at the centers of them. This is reasonable because both the electrostatic potential and the orbital energy are determined from the same wave function.
(11) Zero differential overlap approximation is assumed.
(12) For example, the average D/N values are 0.306 and 0.274 for benzene and cyclohexane, respectively.

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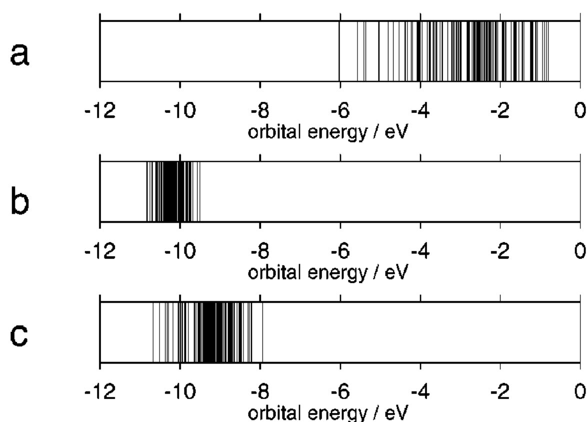

Figure 1. The orbital energies of the lone pairs of the backbone nitrogen atoms. (a) In a vacuum, (b) in water, (c) for the protein in which all of the ionizable residues located on the surface are neutralized.

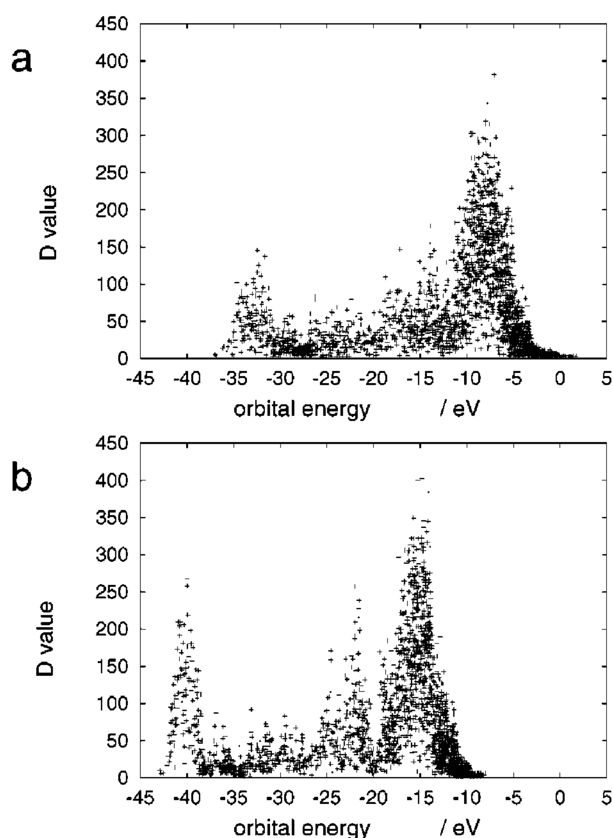

Figure 2. Plot of the *D* value against orbital energy (a) in a vacuum, (b) in water.

As can be seen from Figure 2, the values of the parameter D are always much smaller than the value of N (=3919). This means that each molecular orbital is localized in a relatively compact region in the protein. It is thus of great interest to examine the relationship between the location of each molecular orbital and its electronic property, especially the locations of the frontier orbitals. Table 1 and Figure 3 indicate several molecular orbitals localized on functionally important residues of RNase T_1, which cleaves the P−O5′ ester bond of single-stranded RNA by a transphosphorylation reaction. In solution, HOMO-1 and HOMO-2 are located at Glu 58 and His 40. According to the frontier

Table 1. Molecular Orbitals Localized on the Functionally Important Residues of RNase T_1

	His 40	Glu 58	Arg 77	His 92
in vacuum	HOMO-18	HOMO-10	LUMO+6	LUMO+3
in water	HOMO-2	HOMO-1	LUMO+2	LUMO+3

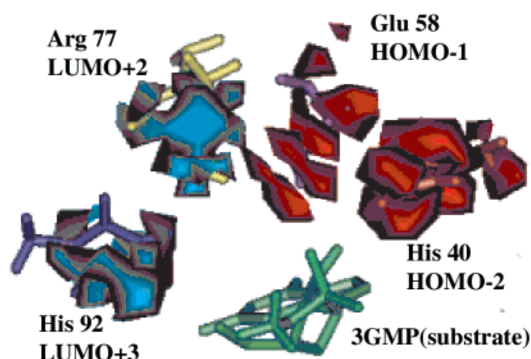

Figure 3. The active site of RNase T_1 and a substrate (3-guanosine 5′-monophosphate). The structure was taken from the PDB data (entry code 4GSP). HOMO-1 and HOMO-2 are represented by red lobes and LUMO+2 and LUMO+3 are by blue lobes.

orbital theory,[13] these residues should be good nucleophilic reagents. In fact, previous experimental studies have indicated that either of them acts as a nucleophilic reagent, abstracting a proton from the 2′-hydroxy group of the substrate[14] LUMO+3 is located at His 92, which acts as an electrophilic reagent donating a proton to the 5′-leaving group.[14] Arg 77, at which LUMO+2 is located, is also believed to play an important role in binding a substrate to the enzyme.[14] Therefore, it can be said that in the solution state the frontier orbitals are located on the functionally important residues. This is in contrast to the situation in the gas phase (see Table 1).

In summary, this study indicated for the first time that the electronic state of the protein undergoes large modifications as a result of hydration. From the calculations presented herein we have arrived at three significant conclusions. First, the dielectric effect of water causes a large lowering of the orbital energies. Second, the interior of a water-soluble protein may be less anisotropic than expected from the heterogeneity of atom packing. Third, the hydration causes the change of relative ordering among the molecular orbitals, resulting in localization of the frontier orbitals at the active site in the case of hydrated RNase T_1. Therefore, the hydration of a protein plays an important role not only in the stabilization of the native structure, but also in the catalytic function.

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