Revisiting the Salt-Induced Conformational Change of DNA with 3D-RISM Theory

Yutaka Maruyama,[†] Norio Yoshida,[†,‡] and Fumio Hirata*[,†,‡]

Department of Theoretical Molecular Science, Institute for Molecular Science, Okazaki 444-8585, Japan, and Department of Functional Molecular Science, The Graduate University for Advanced Studies, Okazaki 444-8585, Japan

Received: December 24, 2009; Revised Manuscript Received: April 4, 2010

The salt-induced conformational transition of DNA is revisited on the basis of the 3D-RISM theory with the purpose of clarifying its physical origin. To take all the contributions to the stability of the molecule into consideration, we performed the optimization of the free energy of B- and Z-DNA in aqueous solutions. Our results exhibited the transition from the B to Z forms with increasing salt concentrations, which is in qualitative accord with the experiments. The results indicate that the transition is caused by an interplay of essentially two contributions, which determine the stability of the molecules, the electrostatic repulsion among charged phosphate groups, and the negative free energy due to counterion binding to those groups. The result is consistent with one of the two models proposed earlier concerning the physical origin of the salt-induced transition of DNA, which attributes the phenomena to the screening of the electrostatic repulsion among phosphate groups, not to the "economy" of hydration, which has been proposed by Saenger et al.

I. Introduction

The salt-induced conformational change of a DNA double helix from its canonical B form to the left-handed Z form[1−3] has attracted a lot of attention from a wide area of science, from physics to physiology.[4−11] The physiology community has been interested in the problem because the left-handed form of DNA is usually found in cancer cells[12,13] and Z-DNA stability may be enhanced by etiological factors related to Alzheimer's disease.[14,15] People in physics and chemistry have been fascinated by the problem, because the molecule belongs to a class of polyelectrolytes which exhibit a variety of interesting physics, including the "counterion condensation."[16−18]

Concerning the physical origin of the salt-induced conformational change, there have been two models, or views, which are quite different from each other. The first of those, which has been proposed by Saenger and co-workers, emphasizes the role of hydration to stabilize the conformations of DNA, especially the hydration of phosphate groups.[19] As the salt concentration increases, the activity of water or the number of water molecules available for hydrating phosphate groups becomes less. Therefore, two phosphates tend to share a water molecule to enhance the efficiency of "hydration." In that respect, the Z-DNA is more favorable, because it has a shorter interphosphate distance due to its characteristic left-handed structure. According to their own terminology, an "economy" of water determines the stability of the two forms of DNA.

The other view to look at the physical origin of the conformational transition is based on the electrostatic repulsion among phosphate groups which are screened by the counterions.[20−29] When the electrolyte concentration is sufficiently low or the screening is weak, the electrostatic repulsion among phosphate groups dominates the stability balance between the two helical forms, which is in favor to B-DNA. On the other hand, when the electrolyte concentration is higher, where the interphosphate repulsion is largely screened, the phosphate counterion interactions dominate the balance, which favors Z-DNA. It is still an open question which model is faithful to the real physics of the conformational transition.

The question may not be answered by any continuum-solvent model that does not account for the hydrogen bonding of water molecules among themselves and with phosphate groups because Saenger's model is related to the bridging of phosphate groups by a water molecule via a hydrogen bond. It is also difficult even for the molecular-simulation methods that include water molecules explicitly. The reason is because the problem requires the sampling of an extraordinarily large configuration space, including water and ions, to obtain the free energy difference of the two conformations of DNA.[30−32]

In the present paper, we report a new method to challenge the problem, which is based on the 3D-RISM theory, a statistical mechanics of molecular liquids.[33] The method does not have any problem of sampling the configuration space of the solution including water and ions because it performs the configuration integral analytically over the entire system, which includes an infinitely large number of solvent molecules. Unlike the continuum-solvent models, the method treats solvent and ion configurations microscopically in terms of the pair correlation functions so that it is capable of accounting for the hydrogen bonding of water molecules among themselves and with phosphate groups of DNA. The method has been applied successfully to a number of problems in biophysics and chemistry to describe the solvation phenomena as well as the molecular recognition of biomolecules in solutions.[34−39] In particular, the robustness of the method was demonstrated recently by comparing the solvation structure of DNA calculated from the theory with that from the molecular dynamics simulation.[40]

II. Computational Method

The energy function that we used in the present work is given by the sum of two terms: the conformational energy, F_{conf}, of

* Corresponding author. E-mail: hirata@ims.ac.jp.
† Institute for Molecular Science.
‡ The Graduate University for Advanced Studies.

10.1021/jp912141u
Published on Web 04/27/2010

the DNA molecule itself and the solvation free energy, $\Delta\mu$, for the interaction of the DNA with the surrounding solvent:

$$F_{\mathrm{tot}} = E_{\mathrm{conf}} + \Delta\mu \tag{1}$$

Here, note that solvation effects were accounted not by the solvation energy, but by the solvation free energy, because we wanted to estimate the average solvation effects for many configurations of water molecules and ions around each fixed solute conformation.

The solvation free energy is calculated by 3D-RISM theory. The detailed formulation of the 3D-RISM theory is provided in ref 33. Here, only a brief interpretation of the theory is provided to help some readers to understand the "physical" aspect of the theory.

Let us consider the average density of water molecules at a position around a DNA molecule. When the position is far from the DNA molecule so as to be regarded as the bulk, the density will be constant, which is the same as in the pure liquid. On the other hand, when it is nearby DNA, the density will be "perturbed" significantly by the field due to the DNA and will be different from that in the bulk, depending on the strength of the perturbation. The 3D-RISM theory can be interpreted as a "nonlinear" perturbation theory as follows.

Let us denote the constant density of solvent atom γ at the bulk, the density nearby DNA, and the density response to the perturbation as ρ, $\rho_\gamma(\mathbf{r})$, and $\Delta\rho(\mathbf{r})$, respectively. Then statement above can be expressed as

$$\rho_\gamma(\mathbf{r}) = \rho + \Delta\rho_\gamma(\mathbf{r}) \tag{2}$$

The density response to the perturbation can be expressed on the basis of the 3D-RISM theory as

$$\Delta\rho_\gamma(\mathbf{r}) = \sum_\alpha \int \chi_{\gamma\alpha}(\mathbf{r}, \mathbf{r}')\, \rho_\gamma\, c_\alpha(\mathbf{r}')\, \mathrm{d}\mathbf{r}' \tag{3}$$

where the $c_\alpha(\mathbf{r}')$ is the "renormalized" perturbation due to the DNA, to which several approximate equations have been devised: for example, the hypernetted-chain (HNC) approximation takes the following expression,

$$c_\gamma(\mathbf{r}) = \exp[-\beta\, u_\gamma(\mathbf{r}) + h_\gamma(\mathbf{r}) - c_\gamma(\mathbf{r})] - [h_\gamma(\mathbf{r}) - c_\gamma(\mathbf{r})] - 1 \tag{4}$$

In the expression, $u_\gamma(\mathbf{r})$ is the direct interaction potential exerted on water molecules from DNA, and $h_\gamma(\mathbf{r})$ is the density fluctuation of solvation at position $\mathbf{r}$, normalized by the bulk density; namely,

$$h_\gamma(\mathbf{r}) = \Delta\rho_\gamma(\mathbf{r})/\rho \tag{5}$$

The three-dimensional distribution function used in this study is defined from $h_\gamma(\mathbf{r})$ by

$$g_\gamma(\mathbf{r}) = h_\gamma(\mathbf{r}) + 1 \tag{6}$$

It is not only the direct interaction $u(\mathbf{r})$ with DNA that perturbs the density of solvent at a certain position, but also that from solvent molecules at the other positions, whose density is also perturbed by the existence of the same DNA. Such "indirect" perturbations are renormalized into the terms including $(h_\gamma(\mathbf{r}) - c_\gamma(\mathbf{r}))$. Such renormalization makes the perturbation highly "nonlinear."

The response function $\chi_{\alpha\gamma}(\mathbf{r}, \mathbf{r}')$ is equivalent to the density pair correlation function of pure (bulk) solvent,

$$\rho^2\chi_{\alpha\gamma}(\mathbf{r}, \mathbf{r}') = \langle \delta\rho_\alpha^{(0)}(\mathbf{r})\, \delta\rho_\gamma^{(0)}(\mathbf{r}')\rangle \tag{7}$$

where $\delta\rho_\alpha^{(0)}(\mathbf{r})$ is the density fluctuation of atom σ in the pure liquid defined by $\delta\rho_\alpha^{(0)}(\mathbf{r}) = \rho_\alpha^{(0)}(\mathbf{r}) - \rho_\alpha$. This response function can be obtained in advance from the one-dimensional RISM theory.

For closing the 3D-RISM equation, we chose the Kovalenko−Hirata (KH) approximation[41,42] for the sake of its advantage of the rapid convergence because the HNC closure shows an extremely poor convergence for the electrolyte solution. The closure takes the form

$$\begin{aligned} g_\gamma^{\mathrm{uv}}(\mathbf{r}) &= \begin{cases} \exp(\chi) \text{ for } & \chi \le 0 \\ 1 + \chi \text{ for } & \chi > 0 \end{cases} \\ \chi &= -\beta u_\gamma^{\mathrm{uv}}(\mathbf{r}) + h_\gamma^{\mathrm{uv}}(\mathbf{r}) - c_\gamma^{\mathrm{uv}}(\mathbf{r}) \end{aligned} \tag{8}$$

where $g_\gamma^{\mathrm{uv}}(\mathbf{r}) = h_\gamma^{\mathrm{uv}}(\mathbf{r}) + 1$ is the 3D solute−solvent site distribution function, $\beta = 1/(k_{\mathrm{B}}T)$, and the interaction potential $u_\gamma^{\mathrm{uv}}(\mathbf{r})$ between solvent site γ.

It is a straightforward task to obtain the one-dimensional distribution or the radial distribution function (RDF), from the 3D-distribution function. The RDFs between position $\mathbf{r}_x$ and solvent γ can be obtained by averaging the 3D-distribution function over the direction around a specified center:

$$g_{x\gamma}(r) = \frac{1}{4\pi}\int g(\mathbf{r}_x + \mathbf{r})\, \mathrm{d}\hat{\mathbf{r}} \tag{9}$$

where $\hat{\mathbf{r}}$ is a direction of vector $\mathbf{r}$, and $\mathbf{r}_x$ indicates a center for the averaging.

We consider NaCl aqueous solution for the solvent and DNA for the solute. The solvation free energy (SFE), $\Delta\mu$, is calculated by using the 3D extension of the Singer−Chandler formula,[43,44]

$$\Delta\mu = \rho k_{\mathrm{B}}T \sum_\alpha \int_{V_{\mathrm{cell}}} \mathrm{d}r \left[\frac{1}{2}(h_\gamma(\mathbf{r}))^2\, \Theta(-h_\gamma(\mathbf{r})) - c_\gamma(\mathbf{r}) - \frac{1}{2}h_\gamma(\mathbf{r})\, c_\gamma(\mathbf{r})\right] \tag{10}$$

Under the isochoric condition, the SFE can be decomposed into the solute−solvent interaction energy, ΔE_{uv}; the water reorganization energy, ΔE_{vv}; and the hydration entropy, ΔS, terms[45]

$$\Delta\mu = \Delta E_{\mathrm{uv}} + \Delta E_{\mathrm{vv}} - T\Delta S \tag{11}$$

The solute−water interaction energy is defined by

$$\Delta E_{\mathrm{uv}} = \sum_\gamma \rho_\gamma \int_{V_{\mathrm{cell}}} u_\gamma(\mathbf{r})\, g_\gamma(\mathbf{r})\, \mathrm{d}\mathbf{r}. \tag{12}$$

For the pair potentials between interaction sites of every species, we employ the common model, which consists of the Lennard-Jones (LJ) and electrostatic interaction terms

$$u_{\alpha\gamma}(r) = 4\varepsilon_{\alpha\gamma}\left[\left(\frac{\sigma_{\alpha\gamma}}{r}\right)^{12} - \left(\frac{\sigma_{\alpha\gamma}}{r}\right)^{6}\right] + \frac{1}{4\pi\varepsilon_0}\frac{q_\alpha q_\gamma}{r} \tag{13}$$

where $\varepsilon_{\alpha\gamma}$ and $\sigma_{\alpha\gamma}$ are the LJ energy and size parameters for a pair of solute site α and solvent site γ, respectively, ε_0 is a dielectric constant of vacuum, and q_α is the partial charge on site α.

To evaluate the optimized structure in solutions, we have to calculate the gradients of solvation free energy with respect to the atomic coordinates of the solute molecule based on the 3D-RISM theory. The analytical expression of the free energy gradient was presented by Sato et al. in the framework of RISM-SCF/MCSCF Theory,[46] and the expression is easily extended to the 3D-RISM formalism.[47] For the 3D-RISM/KH theory, it is expressed as

$$\frac{\partial \Delta\mu}{\partial \mathbf{R}_a} = \sum_\gamma \rho_\gamma \int \mathrm{d}\mathbf{r}\, \frac{\partial u_\gamma^{\mathrm{uv}}(\mathbf{r})}{\partial \mathbf{R}_a} g_\gamma^{\mathrm{uv}}(\mathbf{r}) \tag{14}$$

where $\mathbf{R}_a$ and $u_\gamma^{\mathrm{uv}}(\mathbf{r})$ represent the position vector of solute site a and the interaction potential energy, respectively. The gradient of total free energy of the DNA in solution, eq 1, can be written as

$$\frac{\partial F_{\mathrm{tot}}}{\partial \mathbf{R}_a} = \frac{\partial E_{\mathrm{conf}}}{\partial \mathbf{R}_a} + \sum_\gamma \rho_\gamma \int \mathrm{d}\mathbf{r}\, \frac{\partial u_\gamma^{\mathrm{uv}}(\mathbf{r})}{\partial \mathbf{R}_a} g_\gamma^{\mathrm{uv}}(\mathbf{r}) \tag{15}$$

where the first term of the right-hand side of the equation is calculated analyticaly. We performed the optimization of the DNA structure to minimize the total free energy, F_{tot}, by using a quasi-Newton method with eq 15.

As we stated in the Introduction, the purpose of the paper is to clarify the physical origin of the salt-induced B−Z transition in molecular detail, not to reproduce the experimental results quantitatively. Therefore, we used the potential parameters that have been employed commonly in our previous studies and have reproduced experimental results reasonably well.[34−37,45,48−50] For DNA and ions, we employ the parameters from the Amber-02 force field.[51] The fragments of B- and Z-DNAs with 12 base pairs d(5′CGCGCGCGCGCG3′) were prepared as the solute. We used the SPC/E model[52] with the hydrogen Lennard-Jones term (σ = 1.0 Å and ε = 0.046 kcal/mol) for the water sites.[53] The LJ parameter between unlike atomic sites is determined from the standard Lorentz−Berthelot combination rules. All calculations are carried out for ambient water at temperature T = 298.15 K. The 3D-RISM/KH equations were solved on a grid of 256^3 points in a cubic cell of size 64 Å^3. The grid space of 0.25 Å is fine enough to obtain the results without significant numerical errors. To converge the equations, we used the modified direct inversion in the iterative subspace (MDIIS) method.[54] We prepared several structures of each DNA as initial guesses to avoid being trapped in the local minimum conformation.

III. Results and Discussion

Solvation Structure. The solvent distribution function, $g(\mathbf{r})$, around the B and Z forms of the DNA with the d(5′CGCGCGCGCGCG3′) sequence, which is normalized by the bulk solvent density, is depicted with isosurface plots in Figure 1. The DNA conformations were optimized in water and in aqueous solutions of electrolytes or sodium chloride on the basis of the method described in the previous section. The threshold value for the isosurface plots is set at 3.0, meaning that only $g(\mathbf{r})$'s having values greater than 3.0 are depicted. This is just for the purpose of presentation, and the following arguments are unchanged with the choice of the threshold value employed to draw the figure.

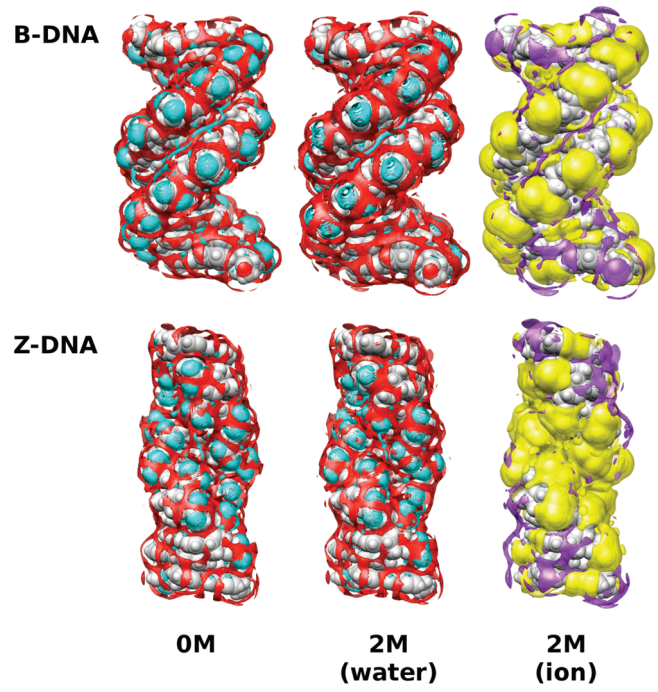

Figure 1. The optimized structures of DNA and the distributions of water oxygen (red), hydrogen (cyan), sodium (yellow), and chlorine (purple) around B- and Z-DNA. The respective density thresholds were 3.0.

The general hydration structure for B-DNA in pure water is essentially the same as that obtained in our earlier study in which we have compared the hydration structure obtained from the 3D-RISM theory with that from the molecular dynamics method. The phosphate groups are covered with a cyan surface, indicating that the group is solvated primarily by the hydrogen atoms of water. Of course, oxygen atoms associated with the hydrogen atoms should be distributed nearby the hydrogen atoms, but they are less distinct so that it cannot be seen in our presentation. In contrast to the phosphate groups, the spaces between the groups are filled by a red surface, indicating that the region is solvated primarily with oxygen atoms of water. The oxygen distribution forms a characteristic network (or train) pattern, a part of which fills the minor groove to make a "spine" hydration. The hydration pattern of Z-DNA is quite different from B-DNA, reflecting the difference in the conformations between the two forms. The phosphate groups are again solvated primarily with hydrogen atoms of water, but in a more crowded manner. A network pattern of water oxygen is observed, which fills the spaces among the phosphate groups. However, such a distinct pattern as the spine hydration is not found.

The distribution of the electrolytes (Na^+ and Cl^-) in 2 mol/L solutions around B-DNA is shown in the upper right picture in the panel. The phosphate groups are covered by a yellow surface, indicating that the groups are primarily solvated with Na^+ ions in the electrolyte solution. The picture suggests that Na^+ ions are bound directly in contact with phosphate oxygen,

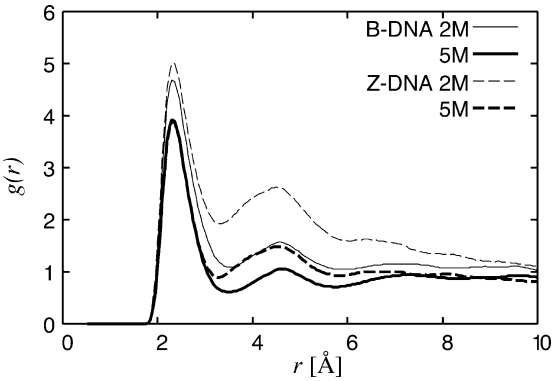

Figure 2. The RDFs between phosphate and sodium: solid lines, B-DNA; dashed lines, Z-DNA; thin lines, 2 M; thick lines, 5 M.

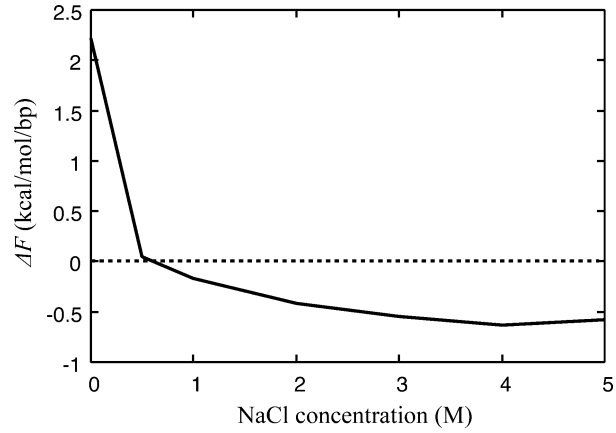

Figure 3. The difference of the total free energy as a function of salt concentration.

not indirectly with intermediate water molecules between the phosphate and ion. On the other hand, the space between the phosphate groups is filled with Cl^- ions (purple color) which shows a pattern somewhat similar to the water oxygen in the water distribution. This also indicates that those water molecules in the major and minor grooves are largely replaced by Cl^- ions in the electrolyte solution.

The distribution of electrolytes around Z-DNA exhibits quite a different pattern from that around B-DNA. The distribution of Na^+ ion is not limited in the vicinity of individual phosphate sites, but extends over the region where the phosphate groups are clustered. The difference in the counterion distribution between the two forms of DNA can be seen more clearly in the radial distribution of Na^+ ions around the phosphate oxygen, which is obtained by taking the average of the 3D-distribution over the orientation of the DNA molecules fixing the O(phosphate)−Na^+ distance.

Shown in Figure 2 is the RDFs for B- and Z-DNA for two different concentrations, 2 mol/L and 5 mol/L. (We have chosen those concentrations to make contact with our earlier study, which has employed the primitive model of an electrolyte solution, but the essential physics is not changed.) The RDF of Na^+ in Z-DNA shows a long-lasting tail that is typical of a polyelectrolyte with a high charge density, whereas that of B-DNA does not show such a long-range behavior in this concentration. The behavior of the RDFs is surprisingly akin to that obtained earlier for a model of polyeletrolyte based on the polymer RISM theory.[28]

Solvation Free Energy and B−Z Transition. Our main results are shown in Figure 3, in which the difference in the free energy of DNAs between the B and Z forms is plotted against the salt concentration. The figure unambiguously shows that the relative stability of the two conformations turns over from the B form to the Z form as the salt concentration is increased. We ignored the contribution from the chain entropy to the difference in the free energy between the two forms of DNA. The result is in qualitative accord with corresponding experiments, although the concentration where the transition takes place is somewhat lower than that in the experimental results. The transition concentration in the experiments is around 2.5 mol/L. We believe that the quantitative difference between the theoretical and experimental results is mainly due to the empirical potential parameters employed in the calculation. Nonetheless, what is important is the fact that the theory could have reproduced the correct trend, which is consistent with the observed one, because this is the first theoretical result due to a molecular theory that is consistent with the experimental finding. There have been several reports,[20−24,26] including one by us,[28] which claimed to have reproduced the experimental results. However, those attempts were based on the continuum solvent model or the primitive model of electrolyte solutions. As we pointed out in the Introduction section, the description based on the continuum solvent model is not capable of answering the question concerning what is the effect of solvent on the salt-induced transition of DNA. On the contrary, our treatment has potential capability to answer the long-lasting question, since it includes solvent water explicitly.

TABLE 1: Total Energy F_{tot} and Its Components (the conformation energy E_{conf} and solvation free energy $\Delta\mu$)[a]

	F_{tot}	E_{conf}	$\Delta\mu$	$\Delta\mu_{water}$	$\Delta\mu_{electrolyte}$
B-DNA 0 M	−423.9	4.3	−428.2	−428.2	−
1 M	−437.3	5.7	−443.0	−10.2	−432.8
2 M	−428.9	6.3	−435.2	11.9	−447.1
3 M	−418.9	6.4	−425.3	26.8	−452.1
4 M	−407.9	6.7	−414.6	39.1	−453.7
5 M	−395.8	7.1	−402.9	49.8	−452.7
Z-DNA 0 M	−421.7	72.5	−494.2	−494.2	−
1 M	−437.4	82.4	−519.8	−18.0	−501.8
2 M	−429.3	83.9	−513.2	12.0	−525.2
3 M	−419.5	84.2	−503.7	29.7	−533.4
4 M	−408.5	85.4	−493.9	43.3	−537.2
5 M	−396.3	85.5	−481.8	55.1	−536.9

[a] $\Delta\mu_{water}$ and $\Delta\mu_{electrolyte}$ are the contributions of solvation free energy from water and salts, respectively. Energy unit is kcal/mol per base pair.

Physical Origin of the B−Z Transition. Shown in Table 1 are the values of free energy (F_{tot}) for both the B- and Z-DNA, including the contributions from the conformational energy (E_{conf}) and those from the solvation free energies due to solvent ($\Delta\mu_{water}$) and salts ($\Delta\mu_{electrolyte}$). A number of observations can be made in the table.

First of all, it is of interest to compare the energetics between B- and Z-DNA just in pure water. (See the first column of Table 1 for each form of DNA) The total free energy including the conformational energy and hydration free energy in pure water is large and negative for both B- and Z-DNA, indicating that both conformations are quite stable in solvent without salts. The reason why it happens is because the contribution from the conformational energy, which is usually positive, is small as compared to that from the solvation free energy. This, in a sense, is puzzling if one considers that the polyelectrolytes carry many charges with the same sign on the phosphate groups, which exert the strong Coulomb repulsion with each other. (See the numbers in parentheses in the third column in Table 1.) However, the puzzle is just apparent, because the Coulomb repulsion among

TABLE 2: The Solute−solvent Interaction Energy and Its Contributions from the Base Pair, Phosphate, and Sugar[a]

	total	base pair	phosphate	sugar
B-DNA 0 M	−1035.8	−119.0	−1178.6	261.8
1 M	−1080.6	−123.3	−1233.6	276.3
	(−208.8)	(−47.2)	(−147.3)	(−14.3)
	[−871.8]	[−76.1]	[−1086.3]	[290.6]
2 M	−1081.2	−123.5	−1233.5	275.8
	(−173.3)	(−43.6)	(−108.4)	(−21.3)
	[−907.9]	[−79.9]	[−1125.1]	[298.6]
3 M	−1079.5	−123.4	−1230.9	274.8
	(−152.8)	(−41.2)	(−87.8)	(−23.7)
	[−926.7]	[−82.2]	[−1143.1]	[298.6]
4 M	−1077.3	−123.2	−1227.9	273.8
	(−137.7)	(−39.3)	(−73.8)	(−24.6)
	[−939.6]	[−83.9]	[−1154.1]	[298.4]
5 M	−1074.7	−122.5	−1225.1	272.9
	(−125.6)	(−37.6)	(−63.5)	(−24.5)
	[−949.1]	[−84.9]	[−1161.6]	[297.4]
Z-DNA 0 M	−1165.8	−120.8	−1310.0	265.0
1 M	−1232.4	−126.1	−1387.6	281.3
	(−222.8)	(−44.2)	(−162.6)	(−16.0)
	[−1009.6]	[−81.9]	[−1225.0]	[297.3]
2 M	−1248.0	−138.9	−1390.4	281.3
	(−184.2)	(−51.8)	(−108.4)	(−24.0)
	[−1063.8]	[−87.1]	[−1282.0]	[305.3]
3 M	−1240.4	−132.4	−1388.4	280.4
	(−145.4)	(−36.3)	(−82.3)	(−26.7)
	[−1088.9]	[−89.9]	[−1306.1]	[307.1]
4 M	−1233.7	−126.0	−1387.3	279.6
	(−127.3)	(−34.2)	(−65.4)	(−27.8)
	[−1106.3]	[91.8]	[−1321.9]	[307.4]
5 M	−1230.7	−125.8	−1383.3	278.4
	(−113.4)	(−32.4)	(−52.7)	(−28.3)
	[−1117.3]	[−93.4]	[−1330.6]	[306.7]

[a] The values in parentheses and square brackets indicate the contributions from water and salts, respectively. The energy unit is kcal/mol per base pair.

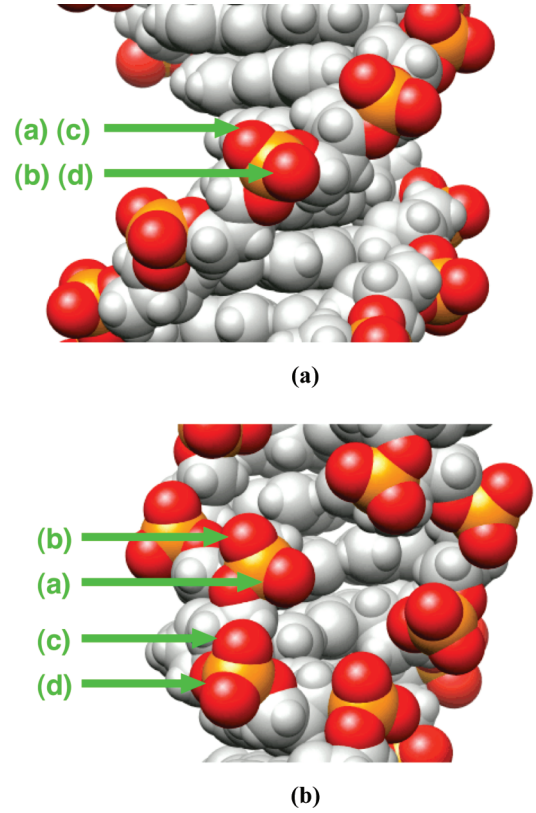

Figure 4. Configurations of oxygen atoms of a phosphate group in (a) B-DNA and (b) Z-DNA. Arrows label the distinctive oxygen atoms of each phosphate group. In B-DNA, a and c are the same, as well as b and d.

the negative charges of phosphate groups is largely screened by positive partial charges in the molecule and also is counterbalanced by the negative contributions, including the interstrand hydrogen bond between the base pairs.

The amount of solvation free energy, which is negative, is greater for Z-DNA than for B-DNA. Similarly, the amount of conformational energy, which is positive, is greater for Z-DNA than for B-DNA. This indicates that Z-DNA is influenced by solvent more seriously than B-DNA, and as a result, the conformation of Z-DNA is distorted more as compared with B-DNA. In any case, it is obvious from Table 1 that the DNA conformation in solution is determined essentially by solvation in pure water.

The conformational energy in solution has large positive values due essentially to the electrostatic repulsion among the negatively charged phosphate groups. The total solvation free energy ($\Delta\mu$) including the contributions from both water and electrolytes, is negative and has much larger absolute values than the conformational energy. This indicates that the conformational stability of DNA is dominated by the DNA-solution (water and ions) interactions. The most remarkable point to be made in the results is that the contributions from solvent and salt completely exchange their positions as the salt concentration increases: for example, $\Delta\mu_{water} = -428.2$ kcal/mol per base pair and $\Delta\mu_{water} = -494.2$ kcal/mol per base pair for B- and Z-DNA, respectively, in pure water, whereas $\Delta\mu_{water} = -10.2$ kcal/mol per base pair and $\Delta\mu_{electrolyte} = -432.8$ kcal/mol per base pair for the 1 mol/L electrolyte solution of B-DNA, and $\Delta\mu_{water} = -18.0$ kcal/mol per base pair and $\Delta\mu_{electrolyte} = -501.8$ kcal/mol per base pair for the 1 mol/L electrolyte solution of Z-DNA. The hydration free energy, $\Delta\mu_{water}$, becomes even positive at a salt concentration higher than 2 M for both B-DNA and Z-DNA. This was absolutely unexpected by us, because water is the most prevalent species that hydrates both the polyelectrolyte and co- and counterions. The result suggests strongly that the phosphates groups of DNA are "solvated" primarily by the counterions, not by water molecules. In other words, the ions are essentially in direct contact with phosphate groups instead of being separated by water molecules.

Then, which group of the DNA makes the most important contribution to the solvation free energy: phosphates, sugar, or base? Shown in Table 2 are the contributions to the solvation energy (the solute−solvent interaction energy), ΔE_{uv}, from each group of DNA. (It should be noted that those are not the solvation "free" energy. $\Delta E_{uv,water}$ changes drastically in the positive direction when the salt concentration is changed from 0 to 1 M, which is parallel to the change in $\Delta\mu_{water}$. However, we believe that it will not make any difference for the essential part of our argument.) As one can see, the contribution from the phosphates is greater by 1 order than that from the other two. The contribution from the sugar group is even positive. The result is indicative that the so-called "spine hydration", which is specifically found in the minor groove of DNA, is playing a minor role in the stabilizing of the conformation of DNA.

An important conclusion drawn from the discussions above is that the stability or the free energy of DNA in an electrolyte solution is dominated by the electrostatic interaction between phosphate groups and the counterions, not by hydration of either phosphate groups or base pairs. The stability of the two forms

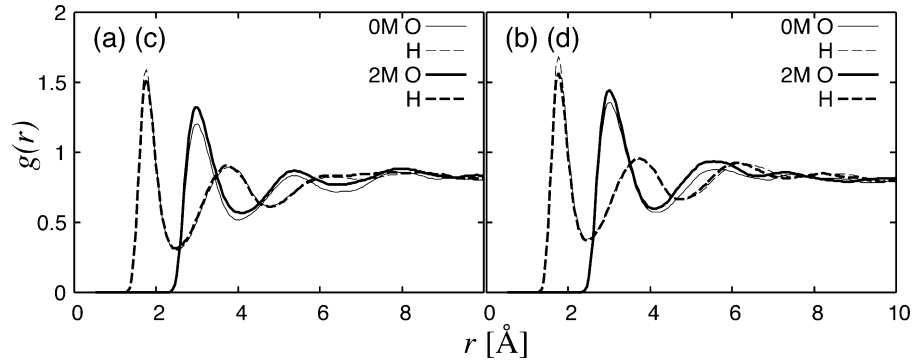

Figure 5. The RDFs between phosphate oxygen and water in B-DNA: solid lines, water oxygen; dashed lines, water hydrogen; thin lines, 0 M; thick lines, 2 M. Each letter in parentheses indicates the oxygen labeled in Figure 4a.

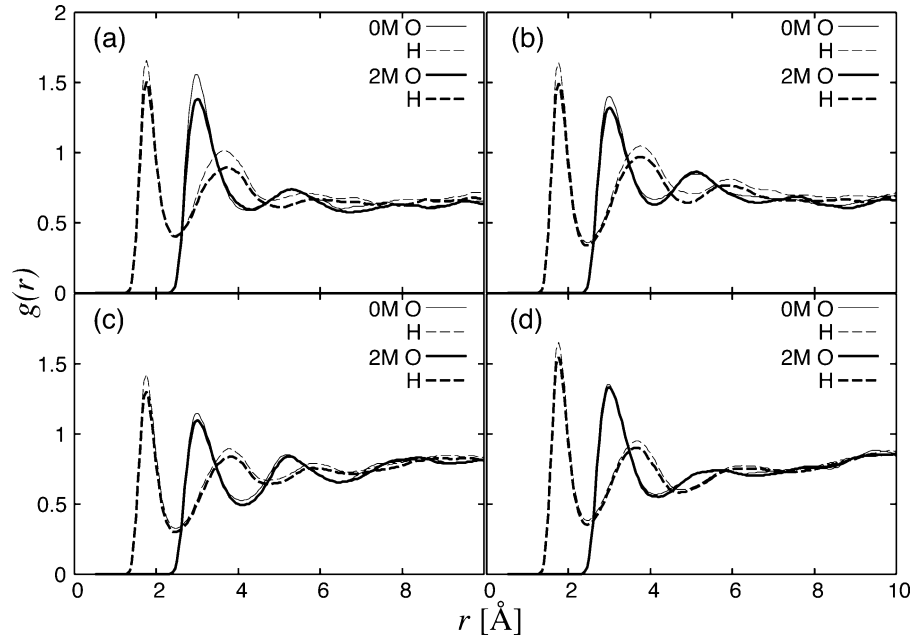

Figure 6. The RDFs between phosphate oxygen and water in Z-DNA: solid lines, water oxygen; dashed lines, water hydrogen; thin lines, 0 M; thick lines, 2 M. Each letter in parentheses indicates the oxygen labeled in Figure 4b.

of DNA crosses over with an increase in the concentration due to the interplay between the Coulomb repulsion among the phosphate groups and the phosphate counterion interactions. In lower concentrations of electrolytes, the difference in stability between the two DNAs is determined by the Coulomb repulsion among phosphates, and the Z form is less stable due to the shorter interphosphate distance in the average. On the other hand, the stability is dominated by the phosphate counterion interactions at higher concentrations, where the Z form becomes more stable as compared with the B form due to the greater phosphate counterion interactions. In any case, our results for the energetics of DNA are inconsistent with the model proposed by Saenger et al. to explain the salt-induced conformational transition of DNA, or "economy of water", because the "hydration" free energy makes a minor contribution to the stability at higher concentration of the electrolytes.

Solvation Structure Around Phosphate Groups. It is of interest to see the solvation structure around phosphate groups in more detail, since the difference in the solvation free energy between the two forms of DNA is an essential cause of the transition. Depicted in detail in Figure 4a, b are the atomic configurations of phosphate groups of B- and Z-DNAs. There are two oxygen atoms in each phosphate group, indicated by arrows in Figure 4, which are freely accessible by either water or ions. Those oxygen atoms are distinct in terms of the pair correlation functions with water or ions because the configurations of DNA atoms surrounding the oxygen atoms are different from each other. In the case of Z-DNA, there are four distinct oxygen atoms due to its characteristic phosphate configuration with a zigzag pattern.

Figure 5 shows the RDFs of water oxygen (O) as well as hydrogen (H) around the two distinct oxygen atoms (a, b in Figure 4a) in B-DNA. The first peak of RDF for hydrogen in the 2 M solution is lower than ones in pure water (0 M). This indicates that ions bind primarily to the oxygen atoms of phosphate groups to replace a water molecule. On the other hand, the first peaks of the oxygen distribution in the 2 M solution are higher than ones in pure water. The peaks are assigned to those water molecules hydrating both the sodium ions and the phosphate oxygen. In Z-DNA, the first peaks associated with hydrogen in the 2 M solutions are lower than ones in pure water (Figure 6). This can be interpreted in the same way as the case of B-DNA. Puzzling is the first peak of RDF for oxygen, which decreases as well in the 2 M solution as compared with the case in pure water. The behavior is opposite the corresponding case of B-DNA. The behavior can be explained by considering the cation distribution around the phosphate oxygen in Z-DNA at 2 M, which is distinctly higher than that in B-DNA (see Figure 7). This indicates that water molecules around a phosphate oxygen are largely replaced by

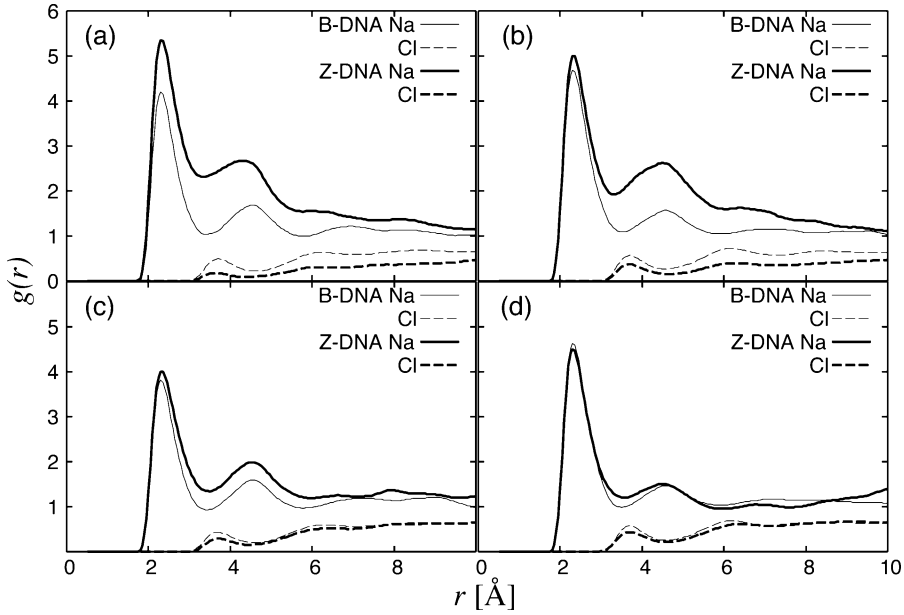

Figure 7. The RDFs between phosphate oxygen and ion in B- and Z-DNA: solid lines, sodium ion; dashed lines, chlorine ion; thin lines, B-DNA; thick lines, Z-DNA. Each letter in parentheses indicates the oxygen labeled in Figure 4.

the cations and that the population of water molecules in the vicinity of a phosphate group is decreased considerably.

The behavior of RDF of water and ions around the phosphate groups refutes again the model proposed by Saenger et al. with respect to the physical origin of the salt-induced conformational transition of DNA. If Saenger's model is correct, we expect to see strengthening of the first peak in RDF between a phosphate group in Z-DNA and a water molecule in a higher concentration due to "bridging" of two phosphates by a water molecule. However, our results show weakening of the first peak in the RDF, which is opposite that expected from the model.

IV. Conclusions

The salt-induced conformational transition of DNA was revisited on the basis of 3D-RISM theory with the purpose of clarifying its physical origin. To take all the contributions to the stability of the molecule into consideration, we performed an optimization of the free energy of B- and Z-DNA in aqueous solutions. Our results exhibited the transition from the B to Z forms with increasing salt concentrations, which is in qualitative accord with the experiments.

The free energy of DNA in electrolyte solutions is dominated by the electrostatic interaction between phosphate groups and the counterions, not by hydration of either phosphate groups or base pairs. The stability of the two forms of DNA crosses over with increasing concentration due to an interplay between the Coulomb repulsion among the phosphate groups and the phosphate counterion interactions. In the lower concentration of electrolytes, the difference in the stability between the two forms of DNA is determined by the Coulomb repulsion among phosphates, and the Z form is less stable due to the shorter interphosphate distance in the average. On the other hand, the stability is dominated by the phosphate counterion interactions in the higher concentration, where the Z form becomes more stable as compared with the B form due to the larger phosphate counterion interactions. In any case, our results for energetics of DNA are inconsistent with the "economy of water " model proposed by Saenger et al. to explain the salt-induced conformational transition of DNA because the "hydration" free energy makes a minor contribution to the stability at higher concentrations of the electrolytes.

The behavior of RDF of water and ions around the phosphate groups also refutes the model proposed by Saenger. If Saenger's model is correct, we should expect to see strengthening of the first peak in RDF between a phosphate group in Z-DNA and a water molecule in higher concentrations, due to "bridging" of two phosphates by a water molecule. However, our results show weakening of the first peak in the RDF, which is opposite that expected from the model.

Acknowledgment. We thank Dr. Komizu for fruitful discussions. This work is supported by a grant from the Grand Challenges in Next-Generation Supercomputing Project, Nanoscience Program, and a Grant-in-Aid for Scientific Research on Innovative Areas "Molecular Science of Fluctuations toward Biological Functions" from the MEXT in Japan. Molecular graphics images were produced using the UCSF Chimera package.[55]

References and Notes

(1) Pohl, F. M.; Jovin, T. M. *J. Mol. Biol.* **1972**, *67*, 375.
(2) Pohl, F. M. *Cold Spring Harbor Symp. Quant. Biol.* **1983**, *47*, 113.
(3) Jovin, T. M.; Soumpasis, D. M.; McIntosh, L. P. *Annu. Rev. Phys. Chem.* **1987**, *38*, 521.
(4) Rich, A.; Nordheim, A.; Wang, A. H. J. *Annu. Rev. Biochem.* **1984**, *53*, 791.
(5) Herbert, A.; Rich, A. *J. Biol. Chem.* **1996**, *271*, 11595.
(6) Gagna, C. E.; Kuo, H. R.; Lambert, W. C. *Cell. Biol. Int.* **1999**, *23*, 1.
(7) Brown, B. A.; Rich, A. *Acta Biochim. Pol.* **2001**, *48*, 295.
(8) Rich, A.; Zhang, S. G. *Nat. Rev. Genet.* **2003**, *4*, 566.
(9) Morange, M. *J. Biosci.* **2007**, *32*, 657.
(10) Wang, G.; Vasquez, K. M. *Front. Biosci.* **2007**, *12*, 4424.
(11) Fuertes, M. A.; Cepeda, V.; Alonso, C.; Pérez, J. M. *Chem. Rev.* **2009**, *106*, 2045–2064.
(12) Boehm, T.; Menglegaw, L.; Kees, U. R.; Spurr, N.; Lavenir, I.; Forster, A.; Rabbitts, T. H. *EMBO J.* **1989**, *8*, 2621.
(13) Thandla, S. P.; Ploski, J. E.; Raza-Egilmez, S. Z.; Chhalliyil, P. P.; Block, A. W.; de Jong, P. J.; Aplan, P. D. *Blood* **1999**, *93*, 293.
(14) Suram, A.; Rao, L. K. S.; Latha, K. S.; Viswamitra, M. A. *Neuromol. Med.* **2002**, *2*, 289.
(15) Hegde, M. L.; Anitha, S.; Latha, K. S.; Mustak, M. S.; Stein, R.; Ravid, R.; Rao, K. S. J. *J. Mol. Neurosci.* **2004**, *22*, 19.

(16) Record, M. T.; Anderson, C. F.; Lohman, T. M. *Q. Rev. Biophys.* **1978**, *11*, 103.
(17) Mannig, G. *Q. Rev. Biophys.* **1978**, *11*, 179.
(18) Fenley, M. O.; Manning, G. S.; Olson, *Biopolymers* **1990**, *30*, 1205.
(19) Saenger, W.; Hunter, W. N.; Kennard, O. *Nature* **1986**, *324*, 385.
(20) Kollman, P.; Weiner, P. *Biopolymers* **1982**, *21*, 1945.
(21) Matthew, J. B.; Richards, F. M. *Biopolymers* **1984**, *23*, 2743.
(22) Pack, G. R.; Klein, B. J. *Biopolymers* **1984**, *23*, 2801.
(23) Frank-Kamenetskii, M. D.; Lukashin, A. V.; Anshelevich, V. V. *J. Biomol. Struct. Dyn.* **1985**, *3*, 35.
(24) Demaret, J. P.; Guéron, M. *Biophys. J.* **1993**, *65*, 1700.
(25) Gavryushov, S.; Zielenkiewicz, P. *Biophys. J.* **1998**, *75*, 2732.
(26) Guéron, M.; Demaret, J. Ph.; Filoche, M. *Biophys. J.* **2000**, *78*, 1070.
(27) Tan, Z.; Chen, S. *Biophys. J.* **2006**, *90*, 1175.
(28) Hirata, F.; Levy, R. M. *J. Phys. Chem.* **1989**, *93*, 479.
(29) Fenley, M. O.; Manning, G. S.; Olson, W. K. *Biopolymers* **1990**, *30*, 1205.
(30) Young, M. A.; Ravishanker, G.; Beveridge, D. L. *Biophys. J.* **1997**, *73*, 2313.
(31) Varnai, P.; Zakrzewska, K. *Nucleic Acids Res.* **2004**, *32*, 4269.
(32) Ponomarev, S. Y.; Thayer, K. M.; Beveridge, D. L. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 14771.
(33) Hirata, F., Ed.; *Molecular Theory of Solvation*; Kluwer: Dordrecht, 2003.
(34) Imai, T.; Hiraoka, R.; Kovalenko, A.; Hirata, F. *J. Am. Chem. Soc.* **2005**, *127*, 15334.
(35) Imai, T.; Hiraoka, R.; Kovalenko, A.; Hirata, F. *Proteins* **2007**, *66*, 804–813.
(36) Yoshida, N.; Phongphanphanee, S.; Maruyama, Y.; Imai, T.; Hirata, F. *J. Am. Chem. Soc.* **2006**, *128*, 12042.
(37) Yoshida, N.; Phongphanphanee, S.; Hirata, F. *J. Phys. Chem. B* **2007**, *111*, 4588.
(38) Ikuta, Y.; Maruyama, Y.; Matsugami, M.; Hirata, F. *Chem. Phys. Lett.* **2007**, *433*, 403.
(39) Maruyama, Y.; Matsugami, M.; Ikuta, Y. *Condens. Matter Phys.* **2007**, *10*, 315.
(40) Yonetani, Y.; Maruyama, Y.; Hirata, F.; Kono, H. *J. Chem. Phys.* **2008**, *128*, 185102.
(41) Kovalenko, A.; Hirata, F. *J. Phys. Chem. B* **1999**, *103*, 7942.
(42) Kovalenko, A.; Hirata, F. *J. Chem. Phys.* **2000**, *112*, 10391.
(43) Singer, S. J.; Chandler, D. *Mol. Phys.* **1985**, *55*, 621.
(44) Kovalenko, A.; Hirata, F. *J. Chem. Phys.* **1999**, *110*, 10095.
(45) Imai, T.; Harano, Y.; Kinoshita, M.; Kovalenko, A.; Hirata, F. *J. Chem. Phys.* **2006**, *125*, 024911.
(46) Sato, H.; Hirata, F.; Kato, S. *J. Chem. Phys.* **1996**, *105*, 1546.
(47) (a) Gusarov, S.; Ziegler, T.; Kovalenko, A. *J. Phys. Chem. A* **2006**, *110*, 6083. (b) Yoshida, N.; Hirata, F. *J. Comput. Chem.* **2006**, *27*, 453.
(48) Phongphanphanee, S.; Yoshida, N.; Hirata, F. *Chem. Phys. Lett.* **2007**, *449*, 433.
(49) Phongphanphanee, S.; Yoshida, N.; Hirata, F. *J. Am. Chem. Soc.* **2008**, *130*, 1540.
(50) Phongphanphanee, S.; Yoshida, N.; Hirata, F. *J. Mol. Liq.* **2009**, *147*, 107.
(51) Cieplak, P.; Caldwell, J.; Kollman, P. *J. Comput. Chem.* **2001**, *22*, 1048.
(52) Berendsen, H. J. C.; Grigera, J. R.; Straatsma, T. P. *J. Chem. Phys.* **1987**, *91*, 6269.
(53) Pettitt, B. M.; Rossky, P. J. *J. Chem. Phys.* **1982**, *77*, 1451.
(54) Kovalenko, A.; Ten-No, S.; Hirata, F. *J. Comput. Chem.* **1999**, *20*, 928.
(55) Pettersen, E. F.; Goddard, T. D.; Huang, C. C.; Couch, G. S.; Greenblatt, D. M.; Meng, E. C.; Ferrin, T. E. *J. Comput. Chem.* **2004**, *25*, 1605.

JP912141U