Peptide Conformations in Alcohol and Water: Analyses by the Reference Interaction Site Model Theory

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Abstract: It is experimentally known that alcohol induces peptides to form α-helix structures much more than water. Though the α-helix structure formed is independent of the alcohol species, degree of the induction increases as bulkiness of the hydrocarbon group in an alcohol molecule increases. In this article we investigate conformations of peptides (Met-enkephalin and the C-peptide fragment of ribonuclease A) in methanol, ethanol, and water using the reference interaction site model theory. Molecular models are employed for the solvents. Our theoretical results show the following. Alcohol indeed facilitates peptide molecules to form the secondary structures with intramolecular hydrogen bonds such as the α-helix. In alcohol a solvophobic atom of a peptide is *less solvophobic* than in water while a solvophilic atom is *less solvophilic*. The solvation free energy in alcohol thus becomes considerably less variable against conformational changes than in water, with the result that the conformational stability in alcohol is governed by the conformational energy. The peptide molecule tends to take a conformation with the lowest conformational energy such as the α-helix, which is independent of the alcohol species. Moreover, these trends are enhanced as bulkiness of the hydrocarbon group in an alcohol molecule increases. In the text, the microscopic origin of the differences between alcohol and water in solvation of peptide molecules, which cannot be obtained by analyses treating the solvent as a dielectric continuum, is discussed in detail.

Introduction

The first-principle prediction of conformations of solute molecules in solvents is one of the most fundamental and essential subjects in modern chemistry. For large, complicated molecules such as peptides and proteins, however, the prediction is a very difficult task. The problem of protein folding, for instance, has long been a central issue in the field but is still unresolved. Conformational transitions, especially those of the secondary structures, in protein molecules are very important aspects in protein folding. An example of great interest is the conversion into non-native β-sheet structures in proteins that cause amyloid diseases.[1−3] Another example is the formation of α-helix structures in the early stage of folding of β-lactoglobulin, the native structure of which is mostly in the β-sheet.[4−6]

Conformations of solute molecules are greatly influenced by the solvent environments, and this is also true for protein molecules. The protein molecule itself tends to take a conformation with the lowest conformational energy. The solvent, on the other hand, forces the protein molecule to take a conformation with the lowest solvation free energy. The protein conformations in solvents are stabilized by competition of these two factors. The solvent effects have been analyzed in detail by treating small peptide molecules in water.[7−9] A significant finding is that in water the solvation free energy for a peptide molecule varies largely from conformation to conformation and remarkably affects the conformational stability. In fact, the peptide conformations stabilized in water are quite different from those in the gas phase. Moreover, addition of salts (e.g., NaCl) to water can alter the conformations to a large extent.[10−12]

Effects of alcohol on peptide and protein conformations[13−20] are very interesting from the standpoints of both the conformational transitions and the solvent effects mentioned above. Melittin and some fragments of β-lactoglobulin, for instance, take extended (unfolded) conformations in aqueous environments, but when alcohol is added, they turn into α-helix structures.[17,19,20] Thus, alcohol induces peptides and proteins to form α-helix structures. Though the α-helix structure formed

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is independent of the alcohol species, the degree of the induction increases as the bulkiness of the hydrocarbon group in an alcohol molecule increases.[20] However, the mechanism of these alcohol effects is still unknown.

In this article, we analyze peptide conformations in methanol and ethanol using the reference interaction site model (RISM) theory,[21−23] a statistical-mechanical theory for molecular fluids. The closure equation employed is of the hypernetted-chain (HNC) type. Met-enkephalin and the C-peptide fragment of ribonuclease A, which were considered in our earlier work,[7−9] are chosen in the analyses. Molecular models are employed for methanol and ethanol. The solvent structures near peptide molecules in different conformations and the solvation free energies are calculated, and the results obtained are compared with those previously obtained for the peptides in water, to elucidate the microscopic origin of the interesting alcohol effects.

Materials and Methods

Peptides Considered. The sequence of Met-enkephalin is Tyr-Gly-Gly-Phe-Met and that of the C-peptide is Lys-Glu-Thr-Ala-Ala-Ala-Lys-Phe-Leu-Arg-Gln-His-Met. A feature of the C-peptide is that five of the residues (Lys-1, Glu-2, Lys-7, Arg-10, and His-12) have groups with large, positive or negative site-charges in their side chains. To make this feature clear, we represent Lys-1, Glu-2, Lys-7, Arg-10, and His-12 by Lys-1^+, Glu-2^-, Lys-7^+, Arg-10^+, and His-12^+, respectively. In the present analyses, we consider peptide molecules in the un-ionized form. The four conformations considered in our previous articles,[7,8] conformations 1 through 4, are revisited for Met-enkephalin. Conformation 1 is the lowest energy conformation in the gas phase and has intramolecular hydrogen bonds. Conformation 4 is one of the conformations stabilized in water and is almost fully extended. It has no intramolecular hydrogen bonds. The two conformations previously treated,[9] conformations 1 and 2, are chosen for the C-peptide. Conformation 1 has the α-helix structure, while conformation 2 is almost fully extended and has no intramolecular hydrogen bonds.

RISM Theory. It is assumed that the solute (peptide) is present in solvent (alcohol or water) at the infinite-dilution limit. The calculation process is then split into two steps where bulk solvent (step 1) and solvent near a solute molecule (step 2) are successively treated. The dielectrically consistent version developed by Perkyns and Pettitt,[24] which is often referred to as the DRISM theory, was employed in our earlier work[7−9] for peptides in water. In the present work for peptides in alcohol, however, the calculations are performed using the RISM theory that was originally developed by Chandler and Andersen[21] and later extended by Hirata and Rossky[22] and Kinoshita and Hirata.[23] A further improved version is the DRISM theory. As long as pure solvent is treated, the results obtained from the RISM and DRISM theories are almost indistinguishable, and the qualitative aspects of our conclusions are not altered.

The basic equations for step 2 comprise the site−site Ornstein−Zernike (SSOZ) relation and the hypernetted-chain (HNC) closure equation. Let the subscripts S and V denote the solute molecule and the solvent molecule, respectively. The solute molecule has m atomic sites ($m = 75$ for Met-enkephalin and $m = 221$ for the C-peptide) and the solvent molecule has n atomic sites. The SSOZ relation in the Fourier space is then expressed by

$$\boldsymbol{\eta}_{SV} = \boldsymbol{w}_{SS}\boldsymbol{c}_{SV}\boldsymbol{H}_{VV} - \boldsymbol{c}_{SV} \tag{1}$$

$$\boldsymbol{\eta}_{SV} = \boldsymbol{h}_{SV} - \boldsymbol{c}_{SV} \tag{2}$$

$$\boldsymbol{H}_{VV} = \boldsymbol{w}_{VV} + \rho_V \boldsymbol{h}_{VV} \tag{3}$$

where $\boldsymbol{H}_{VV}$, $\boldsymbol{\eta}_{SV}$, and $\boldsymbol{w}_{SS}$ are $n \times n$, $m \times n$, and $m \times m$ matrices, respectively, ρ_V is the matrix of the number density of the solvent, $\boldsymbol{h}$ is the matrix of the site−site intermolecular total correlation functions, $\boldsymbol{c}$ is the matrix of the site−site intermolecular direct correlation functions, and $\boldsymbol{w}$ is the intramolecular correlation matrix. $\boldsymbol{H}_{VV}$ is calculated in step 1 and is part of the input data for step 2. The HNC closure equation is given by

$$c_{AB}(r) = \exp\{-u_{AB}(r)/(k_BT) + \eta_{AB}(r)\} - \eta_{AB}(r) - 1 \tag{4}$$

$$A = 1, ..., m; B = 1, ..., n$$

$$\eta_{AB}(r) = h_{AB}(r) - c_{AB}(r) \tag{5}$$

where $u_{AB}(r)$ is the site−site interaction, k_BT has the usual meaning, A is an atomic site in the solute molecule, and B is an atomic site in the solvent molecule.

The solvation free energy for the solute molecule $\Delta\mu_S$ is calculated from[25]

$$\Delta\mu_S/(k_BT) = \sum_{A=1}^{m} \Delta\mu_{SA}/(k_BT) \tag{6}$$

$$\Delta\mu_{SA}/(k_BT) = \int_0^\infty F(r)\, dr \tag{7}$$

$$F(r) = \sum_{B=1}^{n} 4\pi\rho_B r^2 [\{h_{AB}(r)\}^2/2 - c_{AB}(r) - h_{AB}(r)c_{AB}(r)/2] \tag{8}$$

where ρ_B is the number density of atom B. The site−site correlation functions $h_{AB}(r)$ and $c_{AB}(r)$ are calculated by solving the RISM-HNC equations (eqs 1−5). It is convenient to discuss $\Delta\mu_{SA}/(k_BT)$, which depends on the microscopic environment of atom A, as the *apparent* solvation free energy for atom A. For example, as the solvophobicity of atom A increases and/or atom A is less exposed to the solvent, $\Delta\mu_{SA}/(k_BT)$ becomes higher. [For the solvation free energy, the expression that X is higher than Y (Y is lower than X) means $X > Y$.] Hereafter, we refer to $\Delta\mu_{SA}/(k_BT)$ simply as the solvation free energy for atom A.

Model. The site−site interaction $u_{AB}(r)$ has the form

$$u_{AB}(r) = q_Aq_B/r + 4\epsilon_{AB}\{(\sigma_{AB}/r)^{12} - (\sigma_{AB}/r)^6\} \tag{9}$$

$$A = 1, ..., m; B = 1, ..., n$$

where q_A and q_B are the partial charges on site A of the solute molecule and on site B of the solvent molecule, respectively, and the standard combination rule,

$$\epsilon_{AB} = (\epsilon_A\epsilon_B)^{1/2}; \sigma_{AB} = (\sigma_A + \sigma_B)/2 \tag{10}$$

is employed for calculating the Lennard-Jones potential parameters. The potential energy functions and parameters are those based on ECEPP/2 (refs 26−28) and given in our earlier papers.[7,9] For methanol and ethanol molecules, we employ the optimized potentials for liquid

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Table 1. Potential Parameters Employed

atom	σ (nm)	ϵ (kcal/mol)	q (−)
water H	0.0400	0.046	0.4238
O	0.3160	0.156	−0.8476
methanol H	0.0400	0.055	0.4350
O	0.3070	0.170	−0.7000
CH_3	0.3775	0.207	0.2650
ethanol H	0.0400	0.055	0.4350
O	0.3070	0.170	−0.7000
CH_2	0.3905	0.118	0.2650
CH_3	0.3905	0.175	0.0000

Table 2. Solvation Free Energies (kcal/mol) for Conformations 1 through 4 of Met-enkephalin in Water and Methanol

conformation	water	water-0[a]	methanol	methanol-0[a]
1	197	216	73	87
2	178	209	58	82
3	203	229	75	93
4	177	201	59	77

[a] "-0" implies that all the site charges of Met-enkephalin are set to zero.

simulations (OPLS) proposed by Jorgensen.[29] The OPLS parameters are compared with the parameters for the SPC/E water[30] in Table 1. The temperature is set to 298 K. The dimensionless number densities $\rho_V d^3$ ($d = 0.28$ nm) of water, methanol, and ethanol are 0.7317, 0.3246, and 0.2265, respectively. [Just for the C-peptide in water, however, the temperature was set to 273 K and $\rho_V d^3$ is 0.7338 (ref 9). The temperature difference is minor and not likely to alter our conclusions.] "CH_3" and "CH_2" are regarded as single atomic sites, and $n = 3$ and 4 for methanol and ethanol molecules, respectively. It is assumed that all the ethanol molecules take the trans conformations. An important point is that the number density of water is 2.3 times higher than that of methanol (the number density of hydrogen atoms in water is 4.6 times higher than that in methanol), and that of methanol is 1.4 times higher than that of ethanol. Alcohol molecules are larger than water molecules, and this trend is enhanced as bulkiness of the hydrocarbon group in an alcohol molecule increases.

Numerical Method. A sufficiently long range r_L is divided into N mesh points ($r_i = i\delta r$, $\delta r = r_L/N$; $i = 0, 1, ..., N - 1$) and all the functions are represented by their values on these points. The long-range Coulomb potentials are handled in a special manner so that r_L can be minimized.[31] The RISM-HNC equations, a very large set of nonlinear simultaneous equations, are solved by our robust algorithm[31,32] that is over 2 orders of magnitude more efficient than the conventional ones.

Results and Discussion

Met-enkephalin in Water, Methanol, and Ethanol. Table 2 gives the solvation free energies for conformations 1 through 4 of Met-enkephalin in water and methanol. The conformational energies of the four conformations are −12, 12, −3, and 1 kcal/mol, respectively. "Dash zero" implies that all the site-charges of Met-enkephalin are set to zero to shut off electrostatic interaction between the peptide and the solvent (i.e., to make the peptide molecule completely hydrophobic). The absolute values of the solvation free energies in methanol are much smaller than those in water. Even when all the site-charges of the peptide are set to zero, the increase of the solvation free energy in methanol is significantly less than that in water. These trends are enhanced when methanol is replaced by ethanol. For example, the solvation free energies for conformation 4 of Met-enkephalin in ethanol are 51 (full site-charges) and 67 kcal/mol (zero site-charges). The most important feature observed from the table is that in methanol the solvation free energy varies considerably less against conformational changes than in water. The maximum differences among the four conformations in the solvation free energy for the cases of "water", "water-0", "methanol", and "methanol-0" are 26, 28, 17, and 16 kcal/mol, respectively. We emphasize that the conformational stability of a peptide molecule in solvent is governed not by the absolute values of the solvation free energies but by the relative values among different conformations.

Table 3. Solvation Free Energies (kcal/mol) for Some Individual Atoms[a] of Met-enkephalin in Conformation 4: Values in Water, Methanol, and Ethanol

atom	water	methanol	ethanol	water-0[b]	methanol-0[b]	ethanol-0[b]
23 N Gly-2	−1.62	0.96	1.41	3.41	2.22	2.48
56 O Phe-4	−5.98	−3.56	−3.21	2.12	−0.24	−0.72
43 CD1 Phe-4	1.91	−0.23	−0.76	4.68	2.03	1.93
47 CZ Phe-4	2.21	−0.35	−1.00	1.71	−0.60	−1.14

[a] For the definition of the solvation free enrgy for an atom, see eqs 6−8. [b] "-0" implies that all the site charges of Met-enkephalin are set to zero.

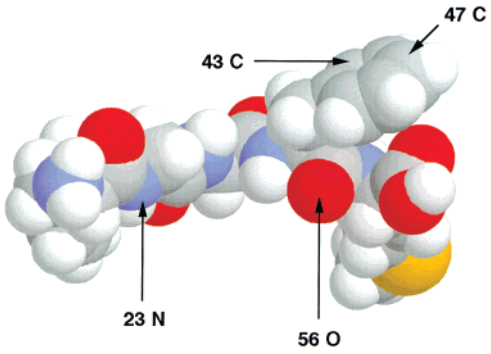

Figure 1. Conformation 4 of Met-enkephalin. "23 N", "43 C", "47 C", and "56 O" represent "23 N" in Gly-2, "43 CD1" in Phe-4, "47 CZ" in Phe 4, and "56 O" in Phe-4, respectively. This figure was created with RasMol.[33]

Table 3 gives the solvation free energies for some individual atoms (for the definition of the solvation free energy for an atom, see eqs 6−8) of Met-enkephalin in water, methanol, and ethanol. Met-enkephalin is in conformation 4. The values calculated with the site-charges set to zero are also included. For the typical hydrophilic atoms with large, negative site-charges in the backbone, "23 N" in Gly-2 and "56 O" in Phe-4 (Figure 1), the values are negative in water, but they become higher in methanol, and even higher in ethanol. For "23 N" which is less exposed than "56 O", the values in alcohol are positive. For the typical hydrophobic atoms in the side chains, "43 CD1" and "47 CZ" in Phe-4 (Figure 1), the values are positive in water, but they become lower in methanol, and even lower in ethanol. A similar feature is observed for the hydrophobic atoms with zero site-charges except "23 N". For "23 N" with zero site-charge, the value in ethanol is higher than that in methanol.

The pair distribution functions $g_{AB}(r)$ (A is an atom of the peptide and B is an atom of the solvent) for $A =$ "23 N" and "56 O" are shown in Figures 2 and 3, respectively. Each figure represents the formation of hydrogen bonding between the peptide atom and solvent oxygen. In particular, for $A =$ "56 O" which is more exposed, the curves for $B =$ H of water, methanol, and ethanol and for $B =$ O of water have sharp peaks. Useful information is obtained from calculation of the coordination number N_B defined by

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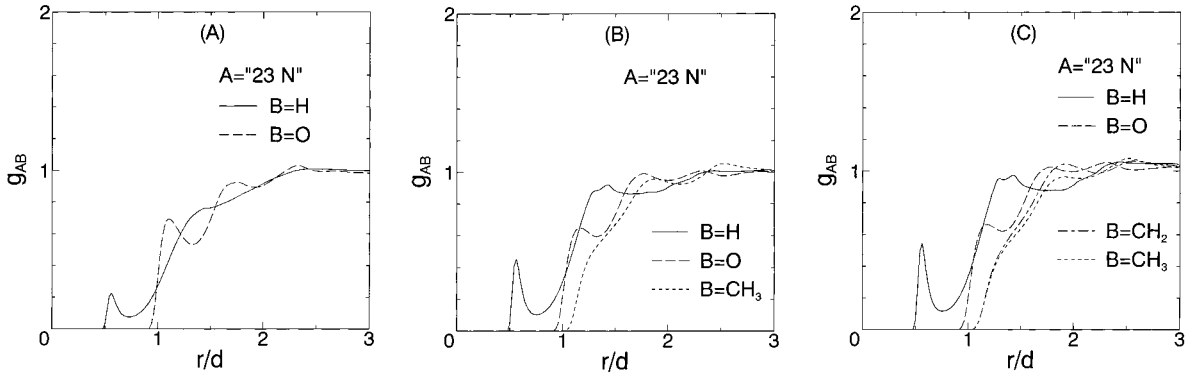

Figure 2. Pair distribution functions $g_{AB}(r)$ for A = "23 N" in Gly-2 of Met-enkephalin immersed in (a) water, (b) methanol, and (c) ethanol.

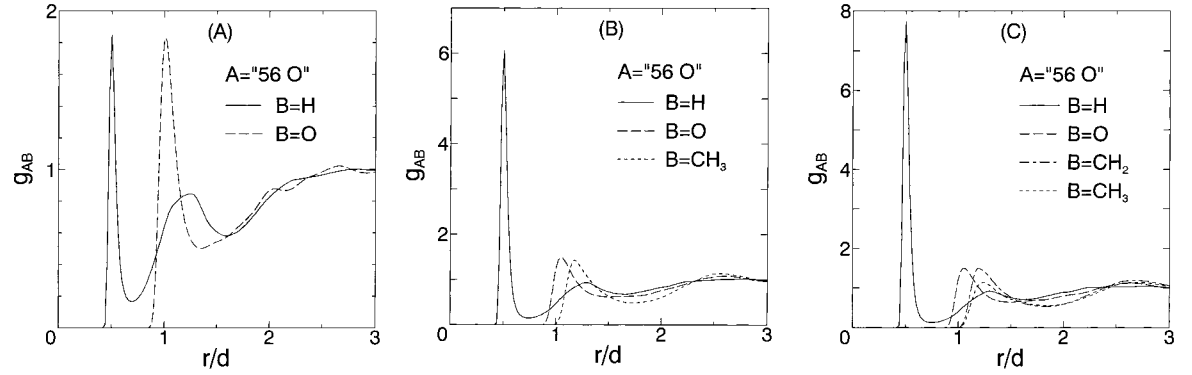

Figure 3. Pair distribution functions $g_{AB}(r)$ for A = "56 O" in Phe-4 of Met-enkephalin immersed in (a) water, (b) methanol, and (c) ethanol.

$$N_B = 4\pi\rho_B \int_0^{r_{\min}} r^2 g_{AB}(r)\, dr \qquad (11)$$

where ρ_B is the number density of atom B and $r_{\min}$ is the position of the first minimum of $g_{AB}(r)$. N_B for B = H gives the average number of solvent-hydrogens forming electrostatic bonding with atom A of the peptide. The values of N_B calculated are given in Table 4. In the last column, the values divided by the value for methanol (i.e., ratios) are given. The N_B values and ratios for methanol are smaller than those for water, and those for ethanol are smaller than those for methanol. This is because the number density of hydrogen atoms is much smaller in methanol than in water and that in ethanol is even smaller. The differences among the water, methanol, and ethanol cases in the N_B values and ratios are larger for "23 N" than for "56 O" (e.g., in the case of B = H of ethanol the ratio for A = "23 N" is smaller than that for A = "56 O"). The reason for this result is the following: "23 N" is less exposed than "56 O", and due to the steric hindrance by the hydrocarbon group in an alcohol molecule, it becomes more difficult for alcohol-oxygen to form hydrogen bonding with "23 N". Since the hydrocarbon group in an ethanol molecule is bulkier than that in the methanol molecule, the steric hindrance effect for ethanol is larger. These results are well reflected on the solvation free energies for "23 N" and "56 O" given in Table 3. The formation of hydrogen bonding between an atom with a large, negative site-charge of the peptide and solvent-oxygen leads to a large decrease in the solvation free energy, but such formation becomes more difficult to achieve in alcohol than in water. This is particularly true for ethanol and for a less exposed atom like "23 N".

We now discuss the solvation free energies for the hydrophobic atoms of the peptide given in Table 3. Alcohol molecules are larger than water molecules and the number density of alcohol is lower than that of water. As a result, in alcohol the work required for the cavity formation is less than that in water, giving rise to lower values of the solvation free energies. This is particularly true for ethanol. There is, however, another significant reason for the result given in the table. The pair distribution functions $g_{AB}(r)$ for A = "47 CZ" are shown in Figure 4 (these functions remain almost unchanged even when the site-charge of "47 CZ" is set to zero). The curves for B = CH_3 of methanol and for B = CH_2 and CH_3 of ethanol have relatively high first peaks. We have calculated the coordination numbers N_B for A = "47 CZ" and B = CH_3 of methanol and B = CH_2 and CH_3 of ethanol. Though the number density of ethanol is lower than that of methanol, the result obtained is the following: N_B(B = CH_3 of methanol) < N_B(B = CH_3 of ethanol) < N_B(B = CH_2 of ethanol). An alcohol molecule has the hydrocarbon group that cannot participate in hydrogen bonding among alcohol molecules, and contact of the hydrocarbon group with a hydrophobic atom of the peptide is significantly stabilized, leading to significant lowering of the solvation free energy. This effect is larger for ethanol than for methanol. As an exception, the solvation free energy for "23 N" with zero site-charge in ethanol is higher than that in methanol. This is because "23 N" is not well exposed and the contact of the hydrocarbon group with the hydrophobic atom is somewhat hindered in ethanol. Table 5 gives the solvation free energy for each residue of Met-enkephalin in water, methanol, and ethanol. Met-enkephalin is in conformation 4. In alcohol, the feature that the solvation free energies for

Table 4. Coordination Numbers of Solvent Hydrogens around Atom A of Met-enkephalin in Conformation 4

A	B	N_B	N_B(ratio)
23 N Gly-2	H of water	0.196	2.20
	H of methanol	0.089	1.00
	H of ethanol	0.072	0.81
56 O Phe-4	H of water	0.883	1.64
	H of methanol	0.538	1.00
	H of ethanol	0.463	0.86

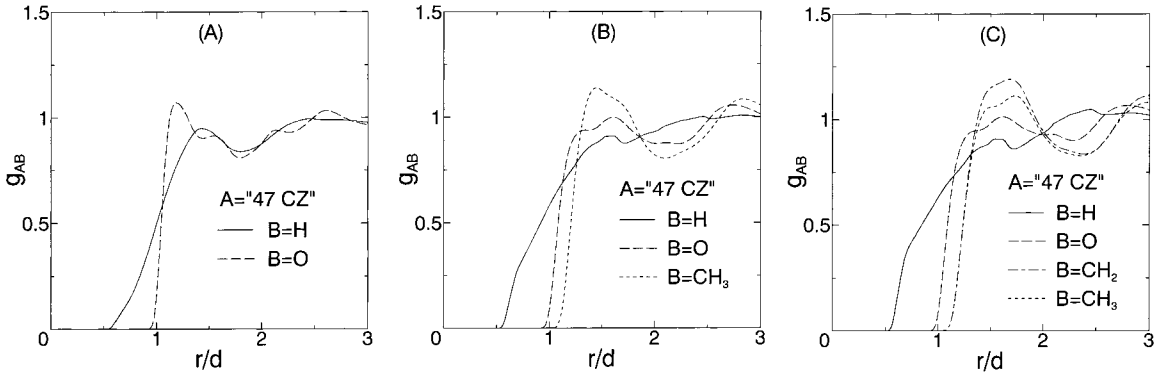

Figure 4. Pair distribution functions $g_{AB}(r)$ for A = "47 CZ" in Phe-4 of Met-enkephalin immersed in (a) water, (b) methanol, and (c) ethanol.

Table 5. Solvation Free Energy (kcal/mol) for Each Residue of Met-enkephalin in Conformation 4: Values in Water, Methanol, and Ethanol

residue	water	methanol	ethanol
Tyr-1	52.9	16.7	13.8
Gly-2	14.7	4.7	4.5
Gly-3	14.2	3.6	3.2
Phe-4	51.1	18.5	16.2
Met-5	44.0	15.9	13.5
total	177	59	51

Table 6. Solvation Free Energies (kcal/mol) for Some Individual Atoms[a] of the C-Peptide in Conforamtion 2: Values in Water and Methanol

atom	water	methanol
17 1HZ Lys-1$^+$	−10.3	−11.3
160 1HH1 Arg-10$^+$	−13.2	−13.8
33 OE1 Glu-2$^-$	−37.0	−29.6
23 O Lys-1$^+$	−7.1	−4.0
119 CD2 Leu-9	2.9	0.9

[a] For the definition of the solvation free energy for an atom, see eqs 6−8.

hydrophobic atoms become lower dominates, and the solvation free energy for each residue becomes considerably lower. This is particularly true for ethanol.

C-Peptide in Water and Methanol. Table 6 gives the solvation free energies for some individual atoms of the C-peptide in water and methanol. The C-peptide is in conformation 2. The atoms with large, positive or negative site-charges in the side chains, "17 1HZ" in Lys-1$^+$, "160 1HH1" in Arg-10$^+$, and "33 OE1" in Glu-2$^-$, are more exposed to the solvent than the carbonyl oxygen in the backbone "23 O" in Lys-1$^+$ (Figure 5). The values for the atoms with large, positive site-charges, "17 1HZ" and "160 1HH1", in methanol are about the same as those in water. However, the values for the atoms with large, negative site-charges, "33 OE1" and "23 O", in methanol are significantly higher than those in water. This is particularly true for the less exposed atom "23 O". For the typical hydrophobic atom, "119 CD2" in Phe-8, the value in methanol is positive but much lower than that in water.

The coordination numbers calculated for the pairs A = "17 1HZ" and B = O, A = "33 OE1" and B = H, and A = "23 O" and B = H are given in Table 7. In the last column, the values divided by the value for methanol (i.e., ratios) are given. The N_B values and ratios for methanol are smaller than those for water, because the number density of methanol is lower than that of water. In the case of methanol, the ratio for the backbone atom is smaller than that for the side-chain atoms. This is because the backbone atom is less exposed and the steric hindrance effect by the hydrocarbon group in a methanol molecule is larger. This result is reflected on the solvation free energies for "23 O" given in Table 6: the value for "23 O" in methanol is considerably higher than that in water. The reason the values for the atoms with large, positive site-charges, "17 1HZ" and "160 1HH1", in methanol are about the same as those in water is as follows. In the case of methanol, when the oxygen atom in a solvent molecule forms electrostatic bonding with "17 1HZ", for example, one hydrogen atom also gets close to "17 1HZ". The hydrogen atom has a large, positive site-charge and interacts with "17 1HZ" through the electrostatic repulsive potential. In the case of water, however, a solvent molecule has two hydrogen atoms, and the effect of the repulsive interaction is larger. As a result, despite the fact that the N_B value (and the ratio) for water is larger, the solvation free energy for "17 1HZ" in water becomes as high as that in methanol. We have considered only methanol for the C-peptide, but we believe that even for the well-exposed atoms with large, positive site-charges discussed above, the solvation free energies in alcohol will eventually become higher as the bulkiness of the hydrocarbon group in an alcohol molecule increases. Table 8 gives the solvation free energy for each residue of the C-peptide in water and methanol. The C-peptide is in conformation 2. In methanol, the solvation free energies for hydrophobic atoms become lower and those for atoms with large, positive site-charges in the side chains are about the same. As a result, the solvation free energies for the residues having groups with large, positive site-charges in the side chains take large, negative values.

Peptide Conformations Stabilized in Alcohol. Since peptide conformations are determined not only by the conformational energy but also by the solvation free energy as mentioned above, it is useful to define the total energy E_T as the sum of the conformational energy E_C and the solvation free energy $\Delta\mu_S$:

$$E_T = E_C + \Delta\mu_S \tag{12}$$

The total energy is an index of the conformational stability of peptides in solvents. Table 9 gives the conformational energies, solvation free energies, and total energies for conformations 1 and 4 of Met-enkephalin. Conformation 1 has intramolecular hydrogen bonds. Conformation 4 is almost fully extended and has no intramolecular hydrogen bonds. $\Delta\mu_{S,W}$, $\Delta\mu_{S,M}$, and $\Delta\mu_{S,E}$ denote the solvation free energies in water, methanol, and ethanol, respectively. $E_{T,W}$, $E_{T,M}$, and $E_{T,E}$ denote the total energies in water, methanol, and ethanol, respectively (for instance, $E_{T,W} = E_C + \Delta\mu_{S,W}$). In terms of the total energy, conformation 4 is more stable by 7 kcal/mol in water, but only by 1 kcal/mol in methanol, and it is as stable as conformation 1 in ethanol. Table 10 gives the conformational energies,

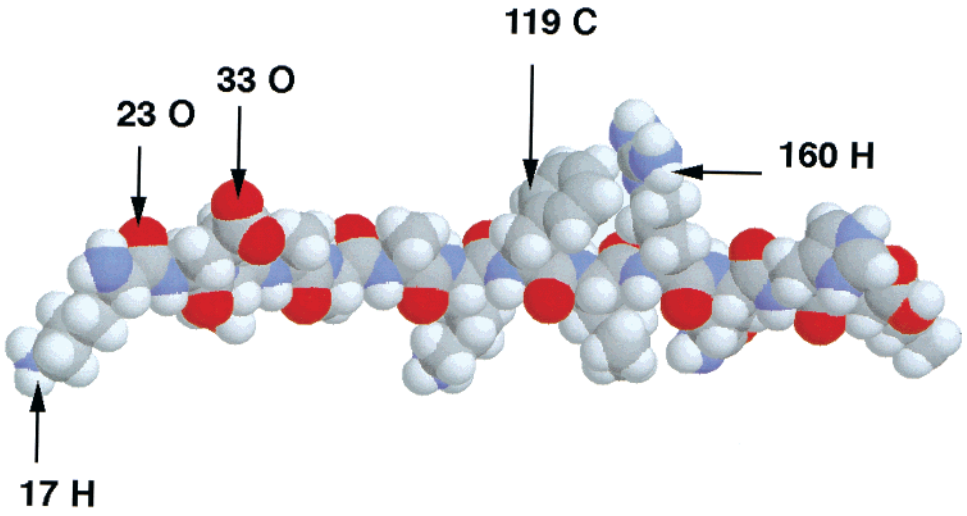

Figure 5. Conformation 2 of the C-peptide. "17 H", "23 O", "33 O", "119 C", and "160 H" represent "17 1HZ" in Lys-1$^+$, "23 O" in Lys-1$^+$, "33 OE1" in Glu-2$^-$, "119 CD2" in Phe-8, and "160 1HH1" in Arg-10$^+$, respectively. This figure was created with RasMol.[33]

Table 7. Coordination Numbers of Solvent Hydrogens or Oxygens around Atom A of the C-Peptide in Conformation 2

A	B	N_B	N_B(ratio)
17 1HZ Lys-1$^+$	O of water	2.69	1.29
	O of methanol	2.08	1.00
33 OE1 Glu-2$^-$	H of water	1.85	1.42
	H of methanol	1.30	1.00
23 O Lys-1$^+$	H of water	0.906	1.61
	H of methanol	0.563	1.00

Table 8. Solvation Free Energy (kcal/mol) for Each Residue of the C-Peptide in Conformation 2: Values in Water and Methanol

residue	water	methanol
Lys-1$^+$	11.3	−20.3
Glu-2$^-$	−30.0	−33.2
Thr-3	28.4	10.2
Ala-4	19.6	7.1
Ala-5	17.9	5.0
Ala-6	20.4	7.8
Lys-7$^+$	−18.3	−44.3
Phe-8	50.0	20.5
Leu-9	48.7	22.3
Arg-10$^+$	1.0	−30.5
Gln-11	36.0	12.6
His-12$^+$	−18.3	−42.3
Met-13	50.8	25.8
total	218	−59

Table 9. Conformational Energies and Solvation Free Energies (kcal/mol) for Conformations 1 and 4 of Met-enkephalin in Water, Methanol, and Ethanol

conformation	E_c	$\Delta\mu_{S,W}$	$\Delta\mu_{S,M}$	$\Delta\mu_{S,E}$	$E_{T,W}$	$E_{T,M}$	$E_{T,E}$
1	−12	197	73	64	185	61	52
4	1	177	59	51	178	60	52

solvation free energies, and total energies for conformations 1 and 2 of the C-peptide. Conformation 1 has the α-helix structure, but conformation 2 is almost fully extended and has no intramolecular hydrogen bonds. The values in water and methanol are given. In terms of the total energy, conformation 2 is more stable by 87 kcal/mol in water, but only by 15 kcal/mol in methanol. Thus, the conformations with intramolecular hydrogen bonds are much more stabilized in alcohol than in water.

Table 10. Conformational Energies and Solvation Free Energies (kcal/mol) for Conformations 1 and 2 of the C-Peptide in Water and Methanol

conformation	E_c	$\Delta\mu_{S,W}$	$\Delta\mu_{S,M}$	$E_{T,W}$	$E_{T,M}$
1	48	457	108	505	156
2	200	218	−59	418	141

In alcohol a solvophobic atom of a peptide is *less solvophobic* (i.e., the solvation free energy for the atom is lower) than in water, and a solvophilic atom is *less solvophilic* (the solvation free energy for the atom is higher). This trend is enhanced as the bulkiness of the hydrocarbon group in an alcohol molecule increases. The solvation free energy in alcohol becomes considerably less variable against conformational changes. As a result, the stability of peptide conformations is governed by the conformational energy. The peptide molecule tends to take a conformation whose conformational energy is as low as possible, i.e., a conformation with intramolecular hydrogen bonds such as the α-helix.

Conclusion

We have analyzed peptide conformations in methanol and ethanol using the RISM-HNC theory. Met-enkephalin and the C-peptide fragment of ribonuclease A, which were considered in our earlier work,[7−9] are chosen in the analyses. The solvent structures near peptide molecules in different conformations and the solvation free energies are calculated, and the results obtained are compared with those previously obtained for the peptides in water. Molecular models are employed for methanol, ethanol, and water. The major conclusions drawn are summarized below.

Alcohol molecules are larger than water molecules and the number density of alcohol is lower than that of water. As a result, the work required for the cavity formation in alcohol is

less. An alcohol molecule has a hydrocarbon group that cannot participate in hydrogen bonding among alcohol molecules, and contact of the hydrocarbon group with a solvophobic atom of a peptide is significantly stabilized. For these reasons, the solvophobic atom of the peptide is *less solvophobic* (i.e., the solvation free energy for the atom is lower) in alcohol than in water. This trend is enhanced as bulkiness of the hydrocarbon group in an alcohol molecule increases: the trend for ethanol is stronger because the hydrocarbon group is bulkier in an ethanol molecule than in a methanol molecule.

There are fewer hydrogen and oxygen atoms per unit volume in alcohol than in water. This property of alcohol and the steric hindrance by the hydrocarbon group cause more difficulty in the formation of electrostatic bonding between a solvophilic atom of the peptide and alcohol-hydrogen or -oxygen. If the solvophilic atom is less exposed, the steric hindrance effect becomes larger. For these reasons, the solvophilic atom of the peptide is *less solvophilic* (i.e., the solvation free energy for the atom is higher) in alcohol than in water, particularly when the atom is not well exposed. This trend is enhanced as the bulkiness of the hydrocarbon group in an alcohol molecule increases.

In alcohol, solvophobic atoms of a peptide can be exposed to the solvent more than in water and exposure of solvophilic atoms becomes less important. The solvation free energy in alcohol becomes considerably less variable against conformational changes. The peptide molecule has a tendency to take a conformation with the lowest conformational energy with intramolecular hydrogen bonds such as the α-helix. We note that the conformation with the lowest conformational energy is independent of the alcohol species. These results are in good agreement with the experimental observations[17,19,20] that can be summarized as follows. Alcohol induces peptides to form α-helix structures, the α-helix structure formed is independent of the alcohol species, and degree of the induction increases as the bulkiness of the hydrocarbon group in an alcohol molecule increases. To the best of our knowledge, this is the first time that the microscopic mechanism of the alcohol effects has been elucidated.

Last, it is worthwhile to add the following. The *β*-sheet structure also has intramolecular hydrogen bonds. For a peptide, if the conformational energy of the *β*-sheet structure is significantly lower than that of the α-helix, alcohol may induce the peptide to form the *β*-sheet. In fact, it was experimentally shown for some peptides[34] that the *β*-hairpin structure linking adjacent strands in an antiparallel *β*-sheet is considerably more stabilized by addition of trifluoroethanol (TFE).

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