Headline Articles

Molecular Dynamics Simulation of Neocarrabiose 2,4′-Bis(sulfate) and Carrabiose 4′,2-Bis(sulfate) as Building Blocks of ι-Carrageenan in Water

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Molecular mechanics and molecular dynamics simulations were performed on two types of disaccharides, neocarrabiose 2,4′-bis(sulfate) and carrabiose 4′,2-bis(sulfate), which are building blocks of ι-carrageenan. Although the conformation and potential-energy surfaces of these disaccharides have been investigated by several researchers, all of them were analyzed using a vacuum calculation. To investigate the conformational behavior of these molecules in water, we performed a molecular dynamics simulation with the presence of explicit water molecules and counter ions around the disaccharides. The trajectories of the glycosidic dihedral angles in the water simulation reproduced the potential-energy surface calculated not with a dielectric constant of 80, but with 1. The electrostatic force was found to act so as to restrict the allowed area of the potential-energy surfaces. The most stable conformation of neocarrabiose 2,4′-bis(sulfate) is $\phi = 162$, $\psi = 174$. Although the global minimum conformation of carrabiose 4′,2-bis(sulfate) is at A1 ($\phi = 176°$ and $\psi = -166°$), the molecular dynamics simulation in water suggested that the structure, B1 ($\phi = 164°$ and $\psi = -87°$), is more stable than A1 by analyzing the trajectory and the hydrogen bonds with water molecules. This indicates that it is important for the conformational analysis to take into account the solvation effect explicitly. The structure of B1 agrees with that found by an NMR experiment.

Carrageenan is a natural polysaccharide which is extracted from seaweed. When its water solution cools down, it is well known that it becomes a gel.[1] The gelation temperature greatly depends not only on the carrageenan concentration, but also on the added salts and their concentrations in the solution. Furthermore, carrageenans have some kinds of species, such as β, κ and ι types, which have different number of sulfuric residues on their galactose ring. The properties of the gel and the gelation mechanism are known to be different from each other. These carrageenans are extensively used as a gelation material and a thickener in such fields as food, cosmetics, and medical supplies by utilizing the differences in the gel properties. However, although many studies have been conducted on the fundamental mechanism of gelation, there still remain many unclear points compared with their widely spread applications being used. According to the results of previous studies, the mechanism of gelation is thought to be as follows. When the water solution cools down, carrageenan makes coil to helix transition; they then aggregate with each other to become a gel. However, it is not clear whether the helical structure is a single or double helix.[2–8] As for the role of the added salt on gelation, it is also not clear. For example, potassium and cesium salts raise the gelation temperature.[2,9] It is considered that these salts would affect the water structure around the sulfuric residue, which relates to gelation through a hydrophobic interaction. However, the details of the microscopic mechanism are still not clear.

Recently, the research of carbohydrates has been developing rapidly because the role of carbohydrate in the signal-transmission mechanism on a cell and the role of the cognition of self and not-self on the cell become clear in the field of biology. As for a full understanding of these biological functions of carbohydrates, an understanding of the conformation in solution should be important. We can see an example in the research of the structure–function relationship in the protein field. However, the research concerning carbohydrate conformation is still being delayed because the experimental methods for the carbohydrate structure are limited in comparison with that of protein research. Among the carbohydrates, carrageenan is one of the molecules whose conformation has been investigated fairly well compared to other carbohydrates. Understanding the conformation of carrageenan is considered to help in our understanding of other carbohydrates that have various biological functions.

We have been investigating the conformation of carrageenan in water using molecular mechanics and molecular dynamics simulations.[10–13] The basic structure of carrageenan is an alternate copolymer of 3,6-anhydro-α-D-galactose and β-D-galactose, which are connected through 1,3 and 4,1 linkages, and called the β-type. The κ-type has one sulfuric residue at the 2-position of the β-D-galactose ring, and the ι-type has another sulfuric residue at the 2-position of 3,6-anhydro-α-D-galactose in addition to the κ-type. There have been some investigations of the conformation of carrageenan by using molecular mechanics and molecular dynamics simulations.[14–16] However, they all investigated the conformation of carrageenan in a vacuum environment. Because the water molecules are known to play an important role in the conformation of carbohydrate, any simulation of carrageenan with explicit water molecules should provide significant information. To examine the effect of hydration on the carrageenan conformation, we have already conducted a molecular dynamics simulation in water on the disaccharide of the building blocks of β-carrageenan.[12,13] The results showed that the stable conformations of the disaccharide in water and in a vacuum are different from each other. Additionally, it was found that there exists a special hydrogen-bonding structure in water, where a water molecule makes a hydrogen bond with hydroxy groups on both galactose rings of the dimer and forms a bridge between two sugar rings. These water bridges would contribute to stabilization of the structure in water.

In this study, as a continuation of the conformational work of the carrageenan family, molecular mechanics and molecular dynamics simulations of ι-type carrageenan were conducted. Iota-carrageenan is an alternate copolymer consisting of 3,6-anhydro-α-D-galactose 2-sulfate (unit A) and β-D-galactose 4-sulfate (unit B); these units are linked through the 1,3 and 4,1 positions on the galactose residues. Therefore, it has two kinds of building blocks of the 1-3 linked A–B dimer, neocarrabiose 2,4′-bis(sulfate), and the 1-4 linked B–A dimer, carrabiose 4′,2-bis(sulfate). In this study, the solution conformations of these two dimer units were examined by analyzing the solvation of the explicit water molecules around these dimers.

Methods

Molecular mechanics and molecular dynamics calculations were performed using the CHARMM program.[17] The method is basically the same as that mentioned in our previous paper.[12] Ha's parameter set for sugar was used in this calculation.[18] The parameter of the sulfuric residue made in our laboratory was added to Ha's parameter set and used in this study. The structures of neocarrabiose 2,4′-bis(sulfate) (A–B dimer) and carrabiose 4′,2-bis(sulfate) (B–A dimer) were taken from fiber X-ray diffraction data of ι-carrageenan,[19] which were obtained from the PDB bank. Reducing and non-reducing ends of the dimers were replaced with hydroxy groups. The nomenclatures of these dimers are shown in Fig. 1. The structures of these dimers are mainly determined by the dihedral angles (ϕ and ψ) of the glycosidic bonds between two sugar rings. The definitions of ϕ and ψ for 1-3 linked A–B dimer are C2–C1–O1–C3′ and C1–O1–C3′-C2′, respectively. In the same way, the definitions of ϕ and ψ for the 1-4 linked B–A dimer are C2′–C1′–O1′–C4 and C1′–O1′–C4–C3, respectively. To search for stable conformations in a vacuum, Ramachandran-like adiabatic potential energy maps were prepared for these dihedral angles using the same minimization-search approach as that employed for previous studies.[12,20] Molecular dynamics simulations in water were performed on a 24.6549 Å length cubic box filled with 482 TIP3P water molecules,[21] the size in which a dimer molecule is fully embedded. The TIP3P water molecules were pre-equilibrated at 300 K. A periodic boundary condition was assigned to the box. Two Na$^+$ ions were initially located 3 Å apart from each sulfur atom of the sulfuric residue as counter ions; they could freely move around within the simulation. Added salts were not used in this simulation. The concentration of the solution was 5.7 wt%. The calculation of the electrostatic potential energy was performed using the Ewald method.[22] A calculation run for a total of 220 ps was made after an equilibration run at 300 K for 20 ps.

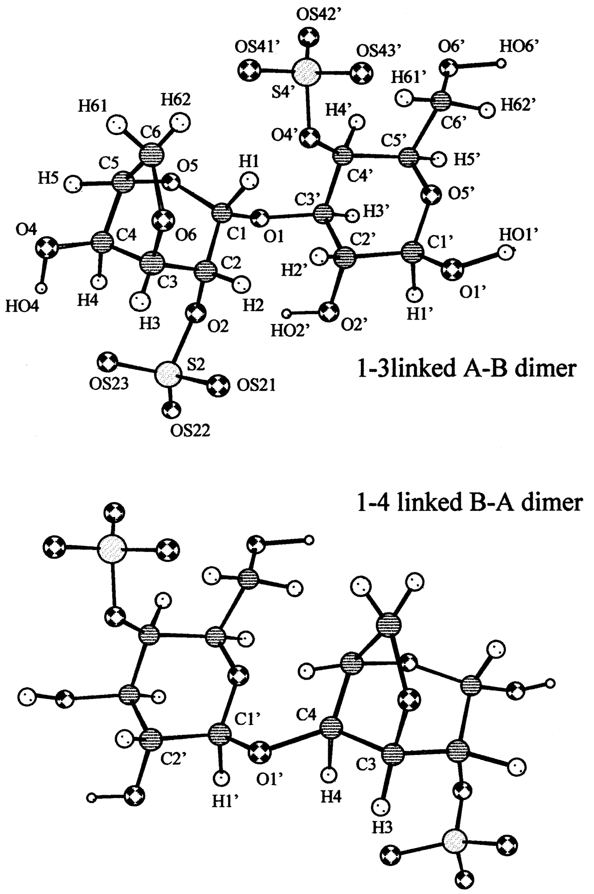

Fig. 1. Nomenclature of the neocarrabiose 2,4′-bis(sulfate) (1-3 linked A–B dimer) and carrabiose 4′,2-bis(sulfate) (1-4 linked B–A dimer).

Results and Discussion

Potential Energy Map of Neocarrabiose 2,4′-Bis(sulfate). An adiabatic potential energy map for neocarrabiose 2,4′-bis(sulfate) (1-3 linked A–B dimer) was obtained (Fig. 2). Because this dimer has one sulfuric residue on each galactose ring, the electrostatic repulsive force between two sulfuric residues should significantly contribute to determining the conformation. An estimation of the effect of such a force is especially important when the molecule is in solution, because the water molecules around the dimer should affect the strength of

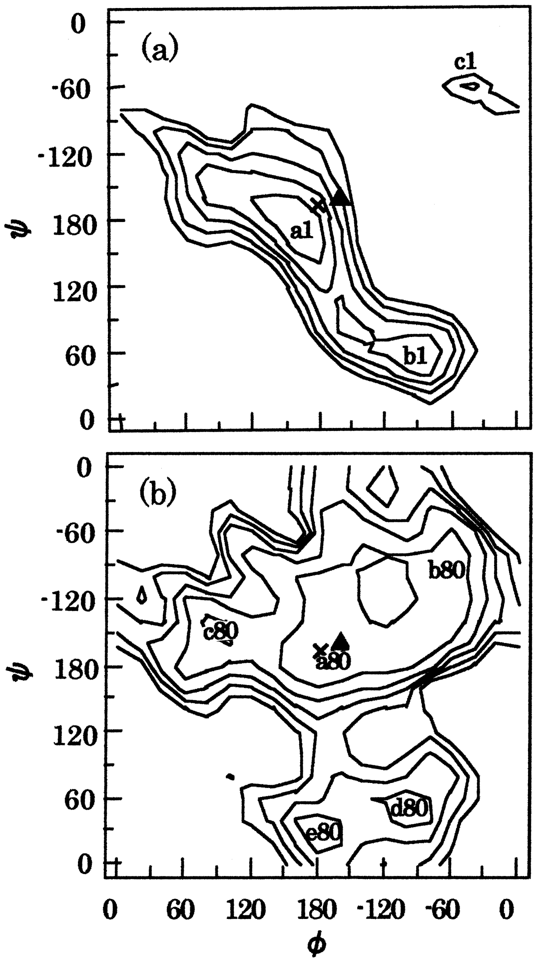

Fig. 2. Potential energy surfaces of neocarrabiose 2,4′-bis(sulfate) calculated by using dielectric constant $\varepsilon = 1$ (a) and $\varepsilon = 80$ (b). Minima positions obtained from $\varepsilon = 1$ and $\varepsilon = 80$ were indicated by a1-c1 and a80-e80, respectively. The global minimum position obtained by Le Questel et al. (×) and the dihedral angles obtained from X-ray diffraction experiment of the ι-carrageenan fiber sample (▲) were also shown.

Table 1. The Dihedral Angles of ϕ and ψ and the Values of Relative Potential Energy at the Minima Obtained for Neocarrabiose 2,4′-Bis(sulfate), Which Is One of the Building Blocks of the ι-Carrageenan. The Wells Obtained for $\varepsilon = 1$ Are Indicated from a1 to c1, and Those Obtained for $\varepsilon = 80$ Are Indicated from a80 to e80, Respectively

		ϕ	ψ	Potential energy
	Well	degrees		kcal/mol
$\varepsilon = 1$				
	a1	162	174	0
	b1	−93	57	2.1
	c1	−48	−55	7.1
$\varepsilon = 80$				
	a80	180	−174	0
	b80	−67	−100	0.5
	c80	85	−146	1.1
	d80	−96	48	2.5
	e80	179	31	2.6

the electrostatic force. However, it is difficult to accurately evaluate the magnitude of the force because the water molecules around the solute change their position and orientation dynamically in solution. Therefore, an adiabatic potential-energy map was made for two cases of the dielectric constant (1 and 80) as an estimation of two extreme cases in water environment. Figure 2a shows that there are three energy minima in the case of a dielectric constant of 1. Similarly, Fig. 2b shows that there are five minima in the case of a dielectric constant of 80. The values of ϕ and ψ at each minimum point and the relative potential energy measured from the value of the global minimum energy are given in Table 1. The global minimum conformation locates at $\phi = 162°$ and $\psi = 174°$ in the case of a dielectric constant of 1, and $\phi = 180°$ and $\psi = -174°$ in the case of a dielectric constant of 80, respectively. This shows that the stable conformation is almost the same in both cases, irrespective of the values of the dielectric constant. However, the low-energy area shown in Fig. 2a can be seen to be more restricted than that in Fig. 2b. Moreover, in the case of $\varepsilon = 80$, there is no high energy barrier among the wells of a80, b80, and c80, and the differences among their relative energies are small. These results indicate that the molecule may possibly move around among these wells rather freely under the circumstance that the electrostatic force is fully shielded. In other words, as was shown in Fig. 2a with $\varepsilon = 1$, the electrostatic force between the sulfuric residues can be said to stabilize the conformation to a more narrow area around the global minimum conformation. Therefore, an evaluation of the shielding effect of the electrostatic force in an actual water environment would be an important factor in determining the conformation of this dimer in aqueous solution. Le Questel et al. calculated the potential-energy map of this molecule in a vacuum.[15] The position of their global minimum conformation was at $\phi = 180°$ and $\psi = -165°$, using our dihedral angle notation. This point is shown in the figure with (×). In their calculation, the Tripos force field and the rigid-residue approximation were used. However, the contribution of the electrostatic force was not taken into account in the calculation. This condition roughly corresponds to our calculation using a dielectric constant of 80, which fully suppresses the electrostatic forces. Stortz and Cerezo[16] obtained the global minimum conformation at $\phi = -168°$ and $\psi = -153°$ by using the MM3 force field with the parameters for sulfate described by Lamba et al.[23] and a dielectric constant of 3. Although the force field and method used are different, the positions of the global minimum conformation are almost the same in all calculations. The point at (▲), $\phi = -163°$ and $\psi = -159°$, shows the dihedral angles which were obtained by an X-ray diffraction experiment using a fiber sample of the iota-carrageenan gel.[19] Although the experiment was conducted with a polymer sample in the gel state, and our calculation of the dimer was made under the condition corresponding to the sol state, it is interesting that the global minimum points are almost the same in all cases irrespective of the different conditions. However, in detail, they are slightly different from our global minimum position at $\varepsilon = 1$, although they still exist in the same potential well. In order to obtain the dynamical behavior in water, the modeling should be done in an environment with explicit water molecules.

Molecular Dynamics Simulation of Neocarrabiose 2,4′-Bis(sulfate) in Water. Molecular dynamics simulations of neocarrabiose 2,4′-bis(sulfate) with two Na^+ counter ions and with explicit water molecules were performed from the minima points shown in Fig. 2. Because the values of the dihedral angles at the minima points of a1, b1, and c1 in Fig. 2a coincide with those of a80, d80, and b80 in Fig. 2b, respectively, it can be considered that the five points from a80 to e80 cover all of the minima points. Therefore, the time histories of the dihedral angles of ϕ and ψ starting from these five minima points were calculated. The obtained trajectories were superimposed on the energy map of $\varepsilon = 80$, as shown in Figs. 3a and b. To avoid any overlapping of the trajectories, they started from a80, b80, and d80, as shown in Fig. 3a; the trajectories starting from c80 and e80 are shown in Fig. 3b. The trajectory of a80, b80, and d80 moved within each initial well at all times investigated. On the other hand, the trajectory which started from c80 initially moved around in the initial well for a while, but then moved toward a80 and stayed there for all of the remaining time. Similarly, the trajectory which started from e80 soon moved toward d80, and never returned to e80. These results indicate that the stable conformations in water should be at three points of a80, b80, and d80. Interestingly, these three points correspond to a1, b1 and c1 obtained with $\varepsilon = 1$, respectively. Therefore, all of the trajectories were next superimposed on the energy map of $\varepsilon = 1$. The results are shown in Fig. 3c. It can be seen that the trajectories well reproduced not only the minimum point, but also the potential shape of the map of $\varepsilon = 1$. Furthermore, as shown in Table 1, the potential energy at c1 is 7 kcal/mol higher than that of the global minimum point at a1. This energy is large enough compared to the thermal energy at room temperature. Although the trajectory started from c1 stayed in the same well in this calculation, this is partly because of the short length of the calculation time, which prevented the trajectory from exceeding the potential barrier from c1 to a1. If a sufficient calculation time could be taken, the transition of the trajectory from c1 to a1 would have been observed. As a summary, the stable structure of the 1-3 linked A–B dimer in water solution would be either a1 or b1, or both.

Figure 3 shows that the trajectory which started from a80 (a1) stayed in the same well within the total time investigated. On the other hand, the trajectory which started from d80 (b1) went around a rather wide area, and part of the trajectory can be seen to travel toward even the mid point between the a1 and b1 wells. In order to investigate the trajectory in more detail, the time courses of the dihedral angles (ϕ and ψ) starting from d80 (b1) are plotted in Fig. 4. The dihedral angles (ϕ and ψ) fluctuate greatly, and they once approach the a1 well at 205 ps, but soon return to the b1 well. This may indicate the possibility of a transition from b1 to a1 well occurring. Therefore, as a check of this possibility for the trajectory to move toward the a1 well from this trajectory, several short molecular dynamics simulations were started from the conformation at a time of 205 ps by randomly assigning the initial velocities. The result showed that the trajectories of all ten trial runs moved toward the a1 well and stayed there. This implies that if a long time simulation could be performed, the transition toward the a1 well would be observed within the occurrence of several trials of trajectories toward the saddle point between the b1 and a1 wells. The above consideration indicates that the conformation of the 1-3 linked A–B dimer in water would take the con-

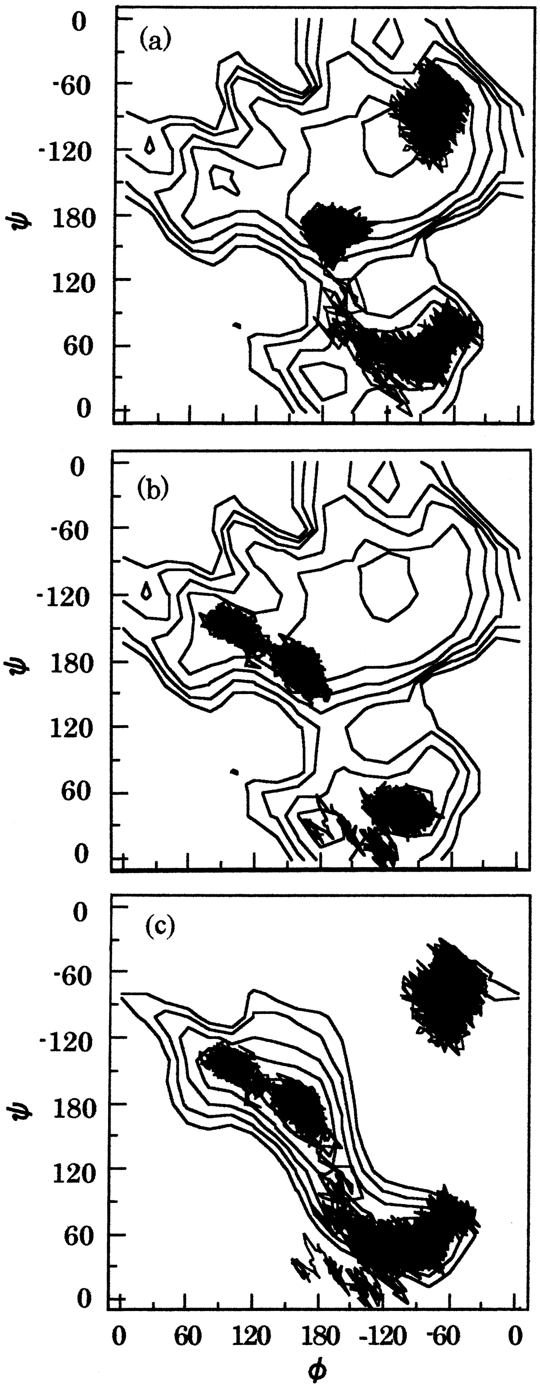

Fig. 3. Trajectories started from a80, b80, and d80 in water and the trajectories started from c80 and e80 were separately superimposed on the potential energy map of $\varepsilon = 80$ in (a) and (b), respectively. All the trajectories started from a80-e80 were superimposed on the potential energy map of $\varepsilon = 1$ in (c).

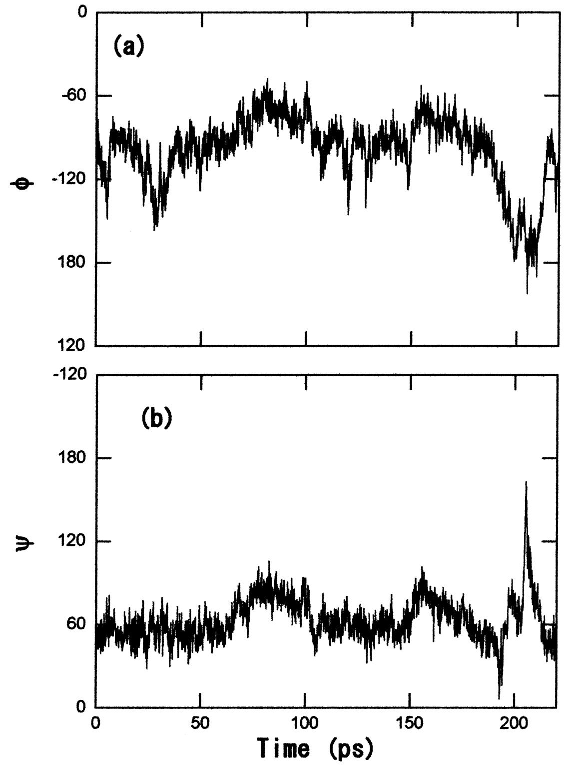

Fig. 4. Histories of the dihedral angles of ϕ and ψ in water simulation started from d80 (b1) conformation.

formation of a1 ($\phi = 162$, $\psi = 174$) as the only stable conformation in an explicit water environment.

Hydration of the 1-3 Linked A–B Dimer. As discussed in a previous paper,[12] the solvation around the carbohydrate molecule would play an important role in determining the conformation in water. In order to investigate the solvation of this dimer, the number of hydrogen bonds formed between water and the disaccharide was calculated and compared between two trajectories starting from the conformations of a80 (a1) and d80 (b1). The average numbers of hydrogen bonds are shown in Table 2. It can be seen that the ether oxygen has roughly one and that the hydroxy oxygen and oxygens of sulfate residues have two to three hydrogen bonds on the average. These values are the same as those previously obtained for other carbohydrates, such as maltose[20] and neocarrabiose.[12] When the total number of hydrogen bonds was compared, the number of hydrogen bonds formed in the a1 conformation was larger than that of b1. Because the hydrogen bonds would stabilize the conformation in water, conformation a1 would be more stable than b1 from an analysis from the hydrogen-bond point of view.

Table 2. Comparison of the Average Number of Hydrogen Bonds Formed to Solvent by Each Sugar Oxygen Atom of Neocarrabiose 2,4′-Bis(sulfate) in a1 and b1 Wells. Hydrogen Bonds Were Calculated Using Geometric Criteria, with a Distance Cutoff of 3.5 Å and an O–H···O Angle Cutoff of 120 Degree

Atom	Number of hydrogen bond		
	"a1" well	"b1" well	Difference
O1	1.27	1.64	−0.37
O2	1.33	0.91	0.42
O4	2.72	1.51	1.21
O5	0.82	1.37	−0.55
O6	1.08	0.97	0.11
OS1	2.75	2.71	0.04
OS2	2.47	2.34	0.13
OS3	2.33	2.20	0.13
O1′	2.76	2.46	0.30
O2′	2.86	1.93	0.93
O4′	0.82	0.80	0.02
O5′	0.88	0.81	0.07
O6′	2.83	3.02	−0.19
OS1′	2.93	2.21	0.72
OS2′	2.23	1.77	0.46
OS3′	2.40	2.36	0.04
total	32.58	29.01	3.57

Table 3. Comparison of the Number of the Water Bridge Formed between the Conformations at a1 and b1 Wells

Water bridge	Percentage of the total trajectory time during which each water bridge occurred	
	"a1" well	"b1" well
O2–O2′	75.5	15.0
O2–OS3′	32.0	—
O5–O2′	14.0	—
O6–OS2′	—	43.0
OS1–O2′	43.5	35.0
OS1–OS3′	15.5	—
OS2–O1′	—	19.0
OS2–O2′	15.5	32.5

The differences in the number of hydrogen bond formed between a1 and b1 are also listed in the same table. The value of the difference at each oxygen atom was small between the two conformations of a1 and b1. However, at the O2′ oxygen, the number of hydrogen bond at a1 is higher than that of b1 by 0.93. This value is large compared to others, except for the O4 oxygen (this oxygen was not considered here because of the end group.) In our previous studies on neocarrabiose, O2′ was found to contribute to the formation of a water bridge, where a water molecule is inserted between two hydroxy oxygens of O2 and O2′ and is connected two galactose rings through hydrogen bond network. Because this type of water bridge acts to stabilize a conformation in water, the existence of the formation of such a water bridge was analyzed in this study for two conformations, a1 and b1. The results are given in Table 3. This shows that the hydrogen-bond bridge between O2 and O2′ was most frequently formed in the a1 conformation, which was 75.5% of the total simulation time. On the other hand, the hydrogen-bond bridges formed in the b1 conformation were all formed with oxygens of sulfate residues. When all of the water bridges were simply summed, the total number in a1 was 56% higher than that in b1. These results indicate that the water bridge would more stabilize the a1 conformation than the b1 conformation. As a conclusion, the stable conformation of

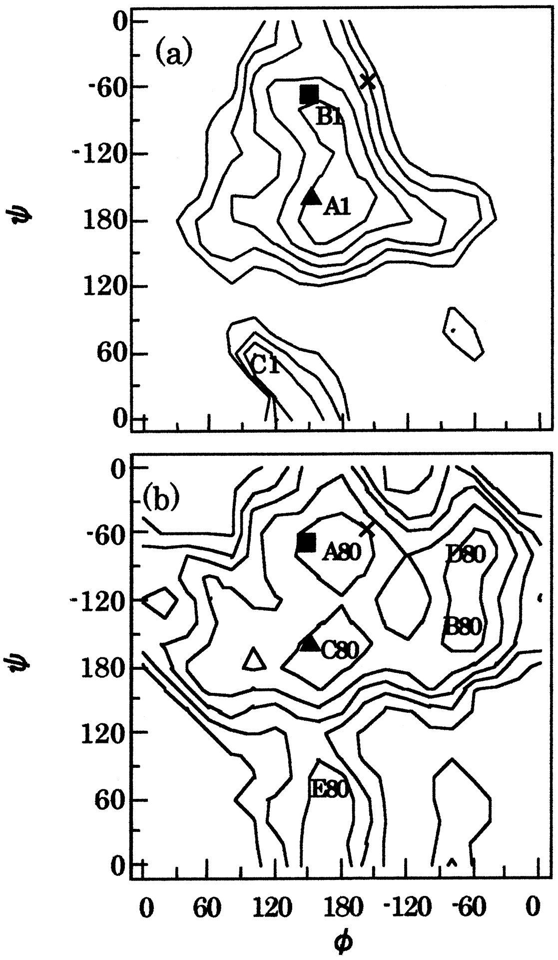

Fig. 5. Potential energy surfaces of carrabiose 4′,2-bis(sulfate) calculated by using dielectric constant $\varepsilon = 1$ (a) and $\varepsilon = 80$ (b). Minima positions obtained from $\varepsilon = 1$ and $\varepsilon = 80$ were indicated by A1-C1 and A80-E80, respectively. The symbols (▲) and (×) were the same as shown in Fig. 2. The symbol (■) was the dihedral angles estimated from NMR experiment by Parra et al.

a 1-3 linked A–B dimer would be a conformation in the a1 well at $\phi = 162$ and $\psi = 174$; this would be only one stable conformation in water.

The presence of Na^+ counterions would also have a possibility to play an important role on the conformation of the dimer molecule. Therefore, the time courses of the movements of Na^+ counterions were investigated. The result indicated that the counterions were not bound in the vicinity of the sulfuric residues, but were rather freely moving around in the surrounding water environment. Moreover, no relational movement could be seen between the time courses of the counterions and the ϕ and ψ dihedral angles. Therefore, the counterions can be considered not to affect the conformation of this dimer in this case. However, the species and the concentration of the added salts are known to affect the conformation of the carrageenan.[2,9] Those effects should be investigated in a future study.

Potential Energy Map and Molecular Dynamics Simulation of Carrabiose 4′,2-Bis(sulfate) in Water. The other building block of ι-carrageenan, carrabiose 4′,2-bis(sulfate) (1-4 linked B–A dimer), was investigated, and a potential energy map of it was calculated for both cases of dielectric constants of 1 and 80. The results are shown in Figs. 5a and b. In the case of $\varepsilon = 1$, three energy minima were found. On the other hand, it can be seen that five potential-energy minima exist in the case of a dielectric constant of 80. The dihedral angles of (ϕ, ψ) and the relative potential energy value at each potential energy minimum are listed in Table 4. The global minimum point was found at A1 ($\phi = 176$, $\psi = -166$) for $\varepsilon = 1$, and at A80 ($\phi = 179$, $\psi = -74$) for $\varepsilon = 80$, respectively. The global minimum point was different, depending on the dielectric constants. However, the difference in the relative energies between A1 and B1 in $\varepsilon = 1$ was only 1.2 kcal/mol, and there is no high energy barrier between these two wells in the map. Similarly, in the case of $\varepsilon = 80$, there are no large differences in energy among A80, B80, C80, and D80, and the map also has no high energy barrier between them. This indicates that the conformation of the 1-4 linked dimer can freely move around the above-mentioned areas. Because the A1 and B1 wells correspond to the C80 and A80 wells, this molecule may be easily moving around between these areas irrespective to the dielectric constant. Le Questel et al. obtained the global minimum structure of this molecule; their result is shown in the same map as (×).[15] Because the atomic charge of the sulfuric residue was not taken into account in their calculation, the dihedral value should be close to our result for $\varepsilon = 80$. Actually, our value obtained for $\varepsilon = 80$ is in good accordance with their value. However, their dihedral angle is located at a slightly higher energy position in our map obtained for $\varepsilon = 1$. On the other hand, the dihedral angles of $\phi = 155$, $\psi = -159$ obtained from an X-ray diffraction experiment of the fiber ι-carrageenan, are shown in the same figure as (▲). This position coincides with the global minimum (A1) obtained for $\varepsilon = 1$, but is different from the global energy minimum position obtained for $\varepsilon = 80$.

In order to investigate the dynamic behavior of this molecule around the conformation of A1 and B1 in water, molecular dynamics simulations were performed; the results are

Table 4. The Dihedral Angles of ϕ and ψ and the Value of Relative Potential Energy at the Minima Obtained for Carrabiose 4′,2-Bis(sulfate), Which Is One of the Building Blocks of the ι-Carrageenan. The Wells Obtained for $\varepsilon = 1$ Are Indicated from A1 to C1, and Those Obtained for $\varepsilon = 80$ Are Indicated from A80 to E80, Respectively

		ϕ	ψ	Potential energy
	Structure	degrees		kcal/mol
$\varepsilon = 1$				
	A1	176	−166	0
	B1	164	−87	1.2
	C1	106	51	3.7
$\varepsilon = 80$				
	A80	179	−74	0
	B80	−62	−143	0.8
	C80	180	−162	0.9
	D80	−62	−82	0.9
	E80	173	69	4.4

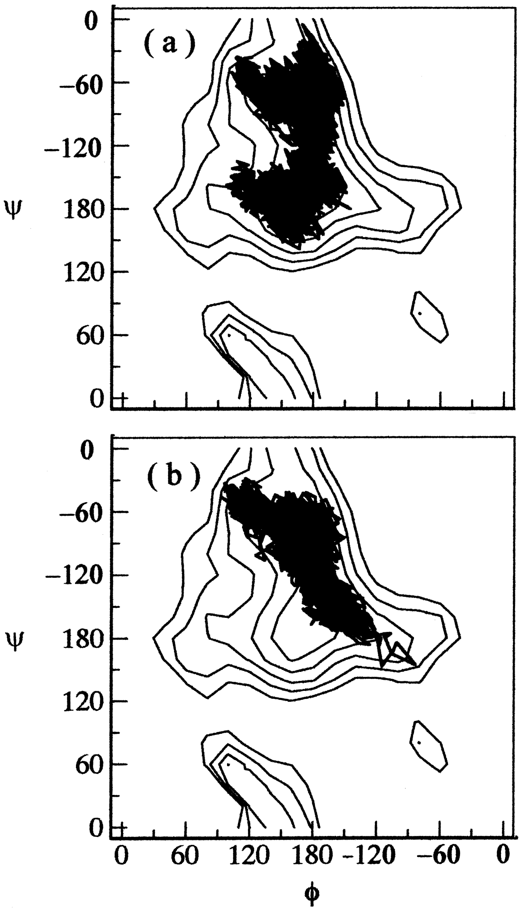

Fig. 6. Trajectories of the carrabiose 4′,2-bis(sulfate) started from A1 (a) and the trajectory started from B1 (b) in water simulation were superimposed on the potential energy map of $\varepsilon = 1$, respectively.

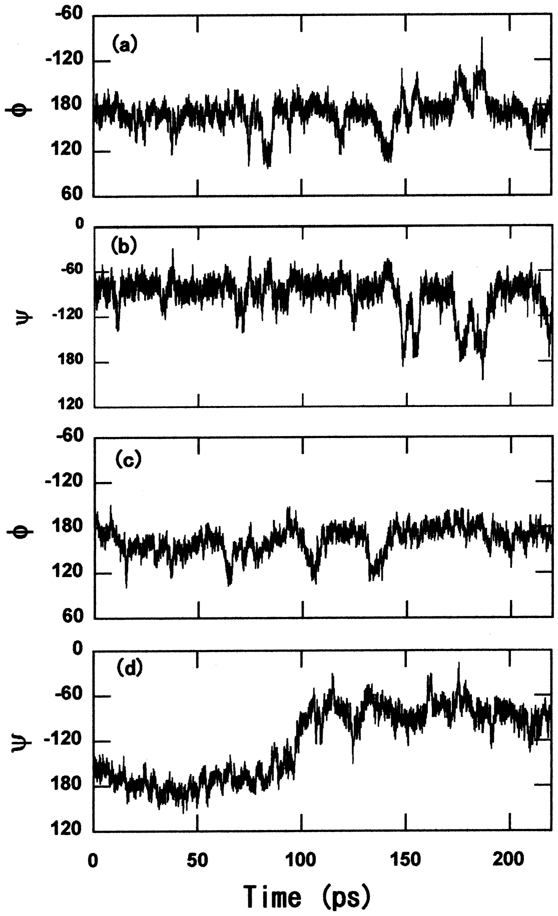

Fig. 7. Time histories of the dihedral angles of ϕ and ψ in water simulation that were started from B1 (a,b) and A1 (c,d).

shown in Fig. 6. The trajectory starting from A1 was superimposed on the ramachandran potential energy map in Fig. 6a, and the trajectory starting from B1 is shown in Fig. 6b. It can again be seen that the trajectories well reproduced the potential shape of the map of $\varepsilon = 1$, as was shown in the 1-3 linked dimer. In order to clear the time course of the trajectories, angles ϕ and ψ in Fig. 6 are plotted against time in Fig. 7. As shown in Figs. 7a and b, the trajectory starting from B1 sometimes moved to the A1 well, but it stayed in B1 most of the time during the simulation. On the other hand, the trajectory starting from A1 stayed in the same well for the first 100 ps, but eventually moved toward the B1 well, and never back to the A1 well within the time investigated, as shown in Figs. 7a and d. This behavior indicates the most provable conformation in water would be the conformation in the B1 well.

The number of hydrogen bonds formed between water and oxygens of the disaccharide were analyzed from the trajectory starting from the A1 well. The trajectory of Figs. 7c and d can be divided into two regions. One is that from 0 to 100 ps; the other is that from 100 ps to 220 ps. These two regions correspond to the A1 and B1 wells, respectively. Therefore, the number of hydrogen bonds formed between water and disaccharide in the A1 and B1 wells was analyzed from these two regions; the results are shown in Table 5. The hydroxy oxygens have roughly two to three, and ether oxygens have one hydrogen bond with water on the average. These are the same as shown in the case of the 1-3 linked A–B dimer. The difference between the two conformations of A1 and B1 are shown in the parenthesis of Table 5. The differences are all small, except for the oxygens of the sulfuric residues. The total value is also almost the same in both conformations. These results indicate that the stability of two conformations of A1 and B1 would almost be the same from an analysis of the number of hydrogen bonds point of view.

The formation of a water bridge was also analyzed; the results are shown in Table 6. It can be seen that the water bridge formed only through the O2′ oxygen in the conformation of the A1 well, and that the largest percentage of formation was between O2′ and OS3 and 58% of the total simulation time. On the other hand, in the B1 conformation, the water hydrogen bond was formed through not only the O2′ oxygen, but also the O6′ oxygen. The sum of all the water bridges would contribute to stabilize the B1 conformation more than A1. As a summary, the stable conformation of carrabiose 4′,2-bis(sulfate) should be in the B1 well, judging from an analysis of the trajectory in water, although the global minimum well in the vacuum potential energy map is the A1 well. The formation of a water bridge may contribute to stabilization of the B1 conformation. However, as was shown in an analysis of the potential-energy

Table 5. Comparison of the Average Number of Hydrogen Bonds Formed to Solvent by Each Sugar Oxygen Atom of Carrabiose 4,2′-Bis(sulfate) in A1 and B1 Wells. The first 0 to 100 ps of the Trajectory Started from A1 Were Used for the Analysis in A1 Well and the Last 100 to 220 ps Were Used for the Analysis in the B1 Well

Atom	Number of hydrogen bond		
	A1 well	B1 well	difference
O1′	1.04	1.06	−0.02
O2′	2.83	2.61	0.02
O3′	2.18	2.13	0.05
O4′	0.96	0.89	0.07
O5′	0.73	0.96	−0.23
O6′	2.83	2.78	0.05
OS1′	2.87	2.13	0.74
OS2′	2.11	1.93	0.18
OS3′	1.93	2.78	−0.85
O1	2.96	2.95	0.01
O2	0.91	0.98	−0.07
O5	1.01	1.07	−0.06
O6	1.31	1.14	0.17
OS1	2.73	2.32	0.41
OS2	2.42	2.68	−0.26
OS3	2.36	2.27	0.09
Total	31.18	30.68	0.50

Table 6. Comparison of the Number of the Water Bridge Formed between the Conformations at A1 and B1 Wells. The First 0 to 100 ps of the Trajectory Started from A1 Were Used for the Analysis in A1 Well and the Last 100 to 220 ps Were Used for the Analysis in the B1 Well

Water bridge	Percentage of the total trajectory time during which each water bridge occurred	
	"A1" well	"B1" well
O2′–O2	1.1	—
O2′–O6	4.5	23.8
O2′–OS1	9.3	5.4
O2′–OS2	1.2	—
O2′–OS3	58.0	3.4
O6′–O2	—	8.7
O6′–OS1	—	30.1
O6′–OS2	—	26.0
O6′–OS3	—	13.9

map in a vacuum and an analysis of the total number of hydrogen bonds, the difference in the stability between the A1 and B1 wells is small. It seems that the conformation of this dimer should stay most of the time in the B1 well, but sometimes fluctuates between the A1 and B1 wells.

Although the experimentally obtained NMR data for neocarrabiose 2,4′-bis(sulfate) cannot be available as far as we know, NMR-NOE data for carrabiose 4′,2-bis(sulfate) was obtained by Parra et al.[24] They observed the inter residue cross-peaks of protons between H1′ and H3, and H1′ and H4. They elucidated the possible conformation of this dimer by using a hard-sphere model of the dimer unit of β-carrageenan, and obtained $\phi = 147^\circ$ and $\psi = -67^\circ$. This point is shown in Fig. 5 as a filled square. It is close to our B1 well. From a model analysis of the conformation B1, the distances between H1′–H3 and H1′–H4 are 2.13 Å and 2.99 Å, respectively, which are sufficiently close to produce the NOE peaks. On the other hand, the distance between H1′–H3 in the A1 conformation is 4.08 Å and H1′–H4 is 2.38 Å, respectively. The distance between H1′–H3 may a little too far to produce a cross peak of NOE. By combining the results of the simulation and the NMR data, the most probable conformation of carrabiose 4′,2-bis(sulfate) would be in B1. Although the NMR data did not predict the possibility of the A1 conformation, it may possible that the conformation sometimes fluctuates toward the A1 well from an analysis of the simulation.

Conclusion

The potential energy map for neocarrabiose 2,4′-bis(sulfate) showed different contour shapes and energy minima according to the dielectric constant used. The trajectory of the molecular dynamics simulation with explicit water molecules indicated that the trajectory reproduced the shape of the map calculated by using dielectric constant 1. The most stable conformation would be a1, which corresponds to dihedral angles of $\phi = 162$, $\psi = 174$. This structure is almost similar to the value of the dihedral angle obtained by an X-ray diffraction experiment of the ι-carrageenan polymer in the gel state. As in our simulation performed on the dimer molecule at room temperature and without any added salts, the experimental conditions may correspond to the sol state. This may indicate that the neocarrabiose 2,4′-bis(sulfate) already takes a similar conformation of the polymer gel of ι-carrageenan even in the sol state.

In the case of the other building block of ι-carrageenan, carrabiose 4′,2-bis(sulfate), there is no big difference in energy between the structure of A1 ($\phi = 176^\circ$ and $\psi = -166^\circ$) and B1 ($\phi = 164^\circ$ and $\psi = -87^\circ$) in the vacuum map. Moreover, the energy barrier between these two wells is not high. Therefore, both of these structures of A1 and B1 would be stable, although the global minimum is at the A1 well. Nevertheless, a molecular dynamics simulation in water showed that the B1 structure is more stable than A1 from an analysis of the trajectory and a hydrogen-bond analysis with explicit water molecules. This indicates that solvation with explicit water molecules is important, and should be taken into account in a conformational analysis of the carbohydrate. The B1 structure also agrees with the distance information obtained by the NMR experiment. However, as for the gel state of the ι-carrageenan polymer, an X-ray diffractive experiment showed that the structure is A1. Although conformation B1 is dominant in the dimer molecule, A1 may be stabilized by the packing effect in the gel state and/or an exclusive volume effect in the polymer state of ι-carrageenan.

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