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Temperature Dependent Self-Diffusion Coefficients of Valinomycin and the Potassium-Valinomycin Complex

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Abstract : Convection effect in liquids has been one of the main targets to be overcome in pulsed-field-gradient NMR measurements of self-diffusion coefficients since the temperature gradient along the sample tube generated by the heating and/or cooling process causes the effect, resulting in additional diffusion. It is known that the capillary is the most appropriate tube type for diffusion experiments at variable temperatures since the narrower tube suppresses convection effectively. For evaluating the properties of hydrogen bonding, diffusion coefficients of the K^+-complexed and free valinomycin in a micro tube have been determined at various temperatures. From the analysis of the obtained diffusion coefficient values, we could conclude that the intramolecular hydrogen bonding in both of the K^+-complexed and free valinomycin in a non-polar solvent is preserved over the observed temperature range, and the temperature dependence of hydrogen bonding is more pronounced in free valinomycin. It is also thought that there is no big change in the radius of the K^+-complexed as temperature is varied, and the ratio of overall radius, $r_{complex}/r_{free}$ is slightly decreased as temperature rises.
Keywords : Diffusion, Valinomycin, Convection, PFG-NMR, Intermolecular hydrogen-bond

INTRODUCTION

The quantitative measurements of self-diffusion coefficient of a liquid by pulsed-field-gradient (PFG) NMR methods have attracted attention because of its easiness and usefulness as a tool for various purpose: mixture analysis,[1] estimation of molecular size,[2,3] intermolecular interactions such as guest encapsulation by supremolecule[4,5] and hydrogen

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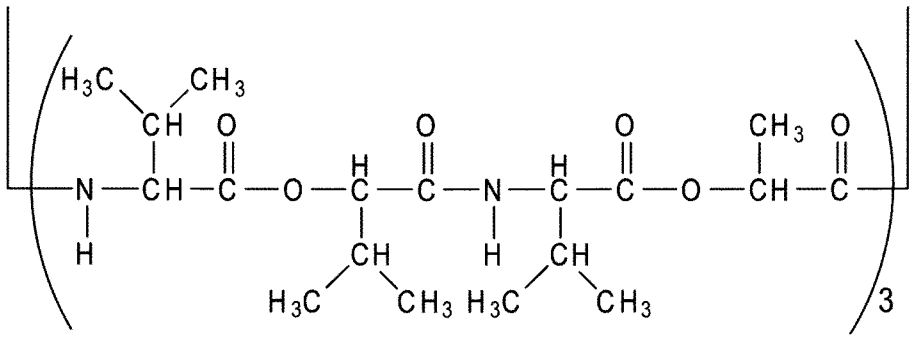
bonding.[6] However, there are usually unavoidable errors in measuring temperature dependent diffusion coefficients using PFG-NMR methods, because cooling or heating probe for maintaining a specific temperature results in spatial and/or temporal inhomogeneities of temperature which lead to fluid convection.[7-9] In general, the errors in diffusion measurements are mainly due to Eddy currents and convection effects. However, it is well known that the effects of Eddy currents generated after gradient pulses could be minimized using appropriate NMR pulse sequences, such as longitudinal eddy-current delay (LED) pulse sequence with bipolar-gradient pulse (BPP) pairs which was used in this experiment too. Therefore, the minimizing of convection effects might be a challenge for obtaining precise diffusion coefficients by PFG-NMR method.

Recently, several methods have been suggested to minimize the convection effects. They include using sample rotation,[10] decreasing sample size,[11] and increasing sample viscosity,[12] etc. One of the various approaches to the problem of convection is using modified NMR tubes,[12] such as Shigemi and capillary tubes. In this work, we have used a micro (capillary) tube to minimize deleterious effects of convection in the self-diffusion measurements at various temperatures.



Valinomycin is a well-known membrane-active antibiotic dodecadepsipeptide which consists of twelve alternating amino acids and esters to form a macrocyclic molecule. It is highly selective for potassium ion relative to sodium ion. Special features of the structure stabilized by hydrogen bonds allow the six ester carbonyl groups in valinomycin to simultaneously interact with the bound potassium ion. Valinomycin acts as a passive carrier for K^+ since it can bind and/or release K^+ when it encounters the membrane surface.

The structure of valinomycin which affects the energy-linked accumulation of alkali ions by mitochondria[13] was determined by several methods such as X-ray crystallography and direct methods.[14-16] The K^+ transfer property of valinomycin at the surface of membrane is strictly correlated with the conformational changes association with ion binding. It is well known that there exists difference in hydrogen bonding between the K^+-complexed and uncomplexed valinomycins. The structure of the K^+-complexed valinomycin has a three-fold symmetry induced by 6 hydrogen bonds between amide protons and amide carbonyl groups. In the uncomplexed form in non-polar solvents, however, all the amino protons are still involved in hydrogen bonding but some of them are linked to ester carbonyl groups instead of amide carbonyls. The difference in hydrogen bonding results in the conformational difference, and finally brings about the differences in the shape and size of molecule.

Recently, Berger *et al.* showed that pulsed-field-gradient (PFG) NMR measurements of self-diffusion could be used to study the strength of hydrogen bonds between different molecules in solution.[17] In principle, the formation of intermolecular hydrogen bond will decrease the diffusion coefficient from that of the non-associated molecule. On the other hand, the formation of intramolecular hydrogen bonding could increase the diffusion coefficient from that expected from its molecular weight especially in relatively large molecules such as peptides or proteins. It can be expected that if the interactions between different parts in a large molecule increase, the size of the molecule should decrease proportionately. Therefore, the measurements of the K^+-complexed and uncomplexed valinomycin's self-diffusion constants can give the supplementary information about their conformational difference induced by the difference in hydrogen bonds.

In this paper, we report the experimental investigation of the self-diffusion coefficients of the K^+-complexed and uncomplexed valinomycins with temperature variation. Based on the results, the correlation between strength of the intermolecular hydrogen bonds and self-diffusion coefficients will also be evaluated as well as the differences in temperature dependences of diffusion coefficients of the K^+-complexed and uncomplexed valinomycins.

EXPERIMENTAL

Micro NMR tubes (ID: ca 1.25 mm) were purchased from Sigma-Aldrich Co. Valinomycin and the deuterated solvents used for our experiment, $CDCl_3$ (100%) were also purchased from Sigma-Aldrich Co. $CDCl_3$ was used without further purification. For the purpose of both of the K^+-complexed valinomycin and free valinomycin being under the same solution conditions such as viscosity, the sample solution was made at the mole ratio of K^+ : valinomycin = 1 : 2 in non-polar $CDCl_3$ solvent. The concentration of valinomycin in the sample solution was 0.0030 M. A conventional 5 mm NMR tube was used for control experiments with the free valinomycin in $CDCl_3$.

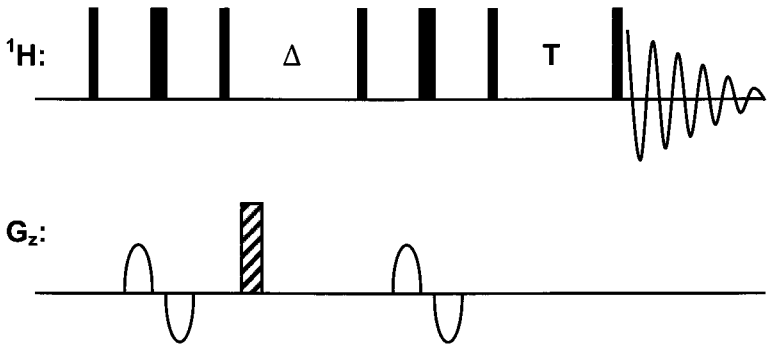


Fig. 1. DOSY-PFG STE sequence with bipolar gradients and a LED (T). Narrow and wide filled rectangles represent the 90° and 180° pulses, respectively. The sine humps and the dashed rectangles represent the ramped gradients and the spoiled gradients.

All NMR data were obtained at various temperatures on a Bruker Avance 600 spectrometer, and diffusion experiments were performed using the bipolar pulse longitudinal eddy current delay pulse sequence (BPPLED)[18] as shown in Fig. 1. The spoiler gradients were also applied at the diffusion period and the eddy current delay to spoil the residual transverse magnetization. Typically, a value of 1-2 ms was used for the gradient duration (δ), ca. 70-130 ms for the diffusion time (Δ), and the gradient strength (g) was varied from ca. 1.5 G/cm to 70 G/cm in 16 steps. Each parameter was chosen to obtain ~90% signal attenuation at the last step experiment. The pulse repetition delay (including

acquisition time) between each scan was 10 s. Data acquisition and diffusion coefficients calculation were performed using the Bruker Topspin software.

RESULTS AND DISCUSSION

The temperature dependency of self-diffusion coefficients for free valinomycin in a conventional 5 mm NMR tube are shown in Fig. 2(a). To magnify hydrogen bonding effects to the structure of valinomycin, a typical non-polar solvent $CDCl_3$ was used as solvent.

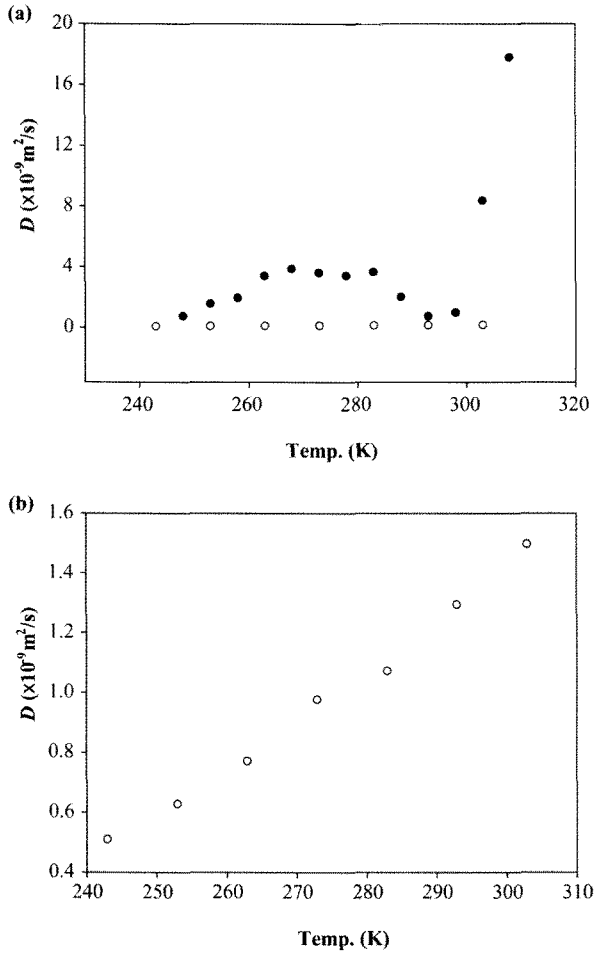


Fig.2. (a) The temperature dependency of the diffusion coefficients for free valinomycin in a 5mm tube (•) and a micro tube (○). (b) The magnified graph for the valinomycin in the micro tube only, for the purpose of showing the temperature dependency clearly.

In general, the diffusion coefficient should be increased as temperature is increased because of the fast molecular motions including diffusion at high temperature. As shown in Fig. 2(a), however, the diffusion coefficient was decreasing when the temperature is increased in some range of 268 K ~ 298 K. This result shows that there exists strong convection of liquid in the 5 mm tube at low temperatures, which is attributed to the temperature gradients along the z-axis of NMR tube due to the inhomogeneity of cooling processes. Since the convection effect depends on the temperature gradient, the diffusion coefficient values must be unreliable especially away from ambient temperature. Additionally, there should be a competition between the convection effects (increasing the diffusion coefficient value) and the substantial reduction of overall molecular mobility (decreasing the diffusion coefficient value) at very low temperature. The V-shaped curve in Fig. 2(a) might be the result of that.

We have also used a micro sample tube for measuring the diffusion coefficients of free valinomycin, and the results are shown in Fig. 2(a) and (b), being compared with the values obtained using the convention 5 mm NMR tube. As expected, the self-diffusion coefficients for the micro tube show gradual increase as temperature raises, while there is large fluctuation in the values of diffusion coefficients for the 5 mm tube with the minimum value at 293 K where temperature regulation is least needed because it is close to ambient temperature. In addition, the magnitude of the obtained value is far smaller in the case of the micro tube. This result confirms that the micro tube is very reliable in diffusion measurements by its suppressing convection effects.[19]

The solution of K^+ and valinomycin dissolved in nonpolar solvent $CDCl_3$ with 1:2 ratio was also probed using the micro tube. Valinomycin, one of antibiotics, is particularly featured by intramolecular hydrogen bonding between its amide hydrogen atoms and oxygen atoms. Norton *et al.* revealed that that there existed six intramolecular hydrogen bonds in both of the K^+-complexed and free valinomycin with a slight difference in conformation which is resulted from the difference in hydrogen bonding.[12] Temperature dependent NMR spectra of the K^+-valinomycin solution obtained in this work support the former conclusion by them. Fig. 3 shows the resonance peaks of two kinds of amide protons of the K^+-complexed and free valinomycin at variable temperatures (243 – 303 K). The chemical shift and coupling constant according to temperature changed significantly for the

amide protons of free valinomycin compared to those of the K^+ complexed valinomycin. This represents that the strength of hydrogen bonding in free valinomycin becomes weaker as temperature rises, whereas intramolecular hydrogen bonding assisted by well-organized structures for the K^+-complexed valinomycin is maintained tightly over the observed temperature range. However the downfield shifts of the amide proton chemical shifts in non-polar solvents, about 1.5 ppm at the same temperature from those in polar solvent such as methanol which is polar and possibly reduces the intramolecular hydrogen bonding, free valinomycin in $CDCl_3$ is apparently in the maintenance of hydrogen bonds even at high temperature. It is known that valinomycin in polar solvents should mainly expose the carbonyl groups to the solvent while in nonpolar solvents such as $CDCl_3$ the iso-propyl groups are located predominantly on the exterior of the molecule.

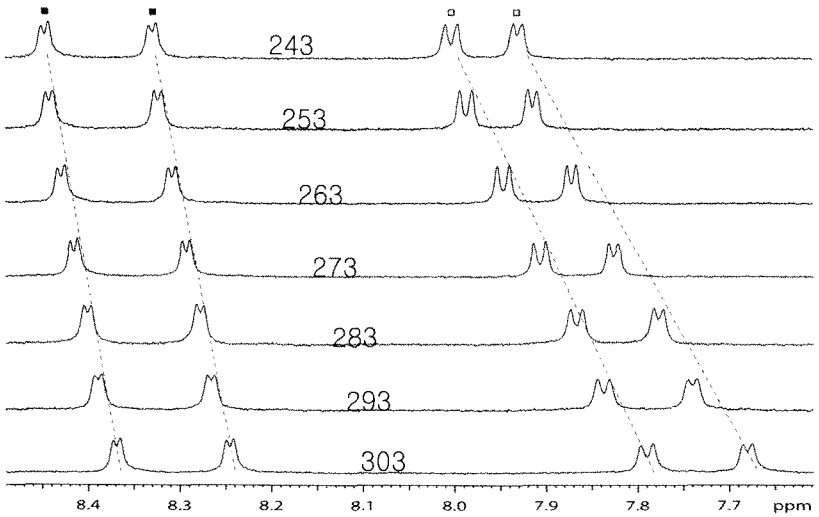


Fig.3. Partial ^1H NMR spectra of K^+ : valinomycin = 1:2 solution (■ : amide protons of the K^+-complexed valinomycin, □ : amide protons of free valinomycin) at variable temperatures.

To estimate and compare the overall molecular size of the K^+-complexed and free valinomycin, the Stokes-Einstein equation was used,

$$D = \frac{k_B T}{6\pi\eta r_s}$$

where r_s is the van der Waals radius of the molecule, k is the Bolztmann constant, T is the temperature, η is the viscosity of the solution and D is the self-diffusion constant. In this study, the Stokes-Einstein equation could be useful since it assumes spherical molecules much larger than the solvent molecules.

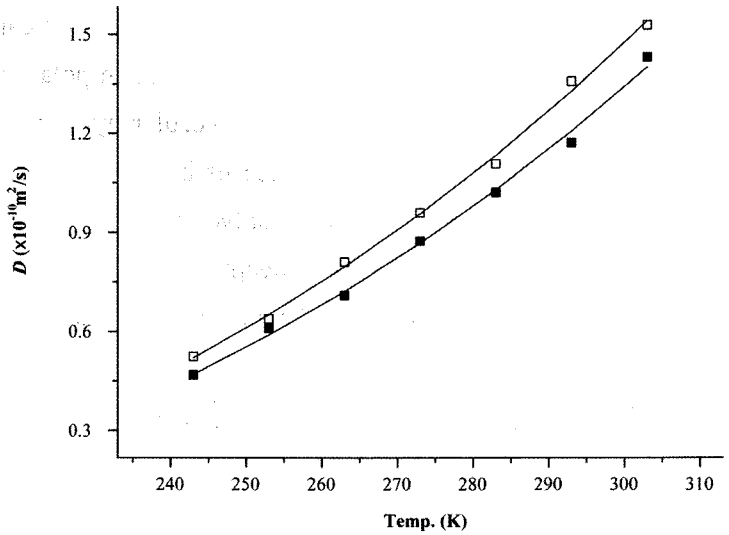


Fig.4. The temperature dependency of the diffusion coefficients of the K^+-complexed valinomycin (■) and free valinomycin (□) in a micro tube.

The diffusion coefficients for the K^+-complexed and free valinomycin were also acquired at various temperatures (243 – 303 K, see Fig. 4), and their values were 1.43 x10^{-10}(complex) / 1.53 x10^{-10} m^2s^{-1} (free) at 303 K and 4.69 x10^{-11}(complex) / 5.24 x10^{-11} m^2s^{-1} (free) at 243 K. According to the Stoke-Einstein equation, in principle, $D_{free}/D_{complex}$ can represent $r_{complex}/r_{free}$. The ratios of molecular radius $r_{complex}/r_{free}$ which were calculated by experimentally determined self-diffusion constants were 1.07 and 1.12 at 303K and 243K, respectively. The fact that the value of $r_{complex}/r_{free}$ at high temperature is smaller than that at low temperature means that the relative radius of free valinomcin is larger at high temperature than that at low temperature. This coincides with the above interpretation of temperature dependent ^{1}H 1D spectra that the hydrogen bonding in free valinomycin loses its character more quickly than that in the K^+-complexed valinomycin, because weakening of the intramolecular hydrogen bonds can result in enlarging the size of molecule.

In summary, we have demonstrated that the micro tube is very reliable for the determining self-diffusion coefficients by PFG-NMR. We have also extracted the

information about the structure difference between the K^+-complexed and free valinomycin induced by the difference in intramolecular hydrogen bonding by evaluating the temperature dependence of self-diffusion coefficient values. It shows that the diffusion measurement by PFG-NMR could be useful to examine the properties of intramolecular hydrogen bonding.

Acknowledgement

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