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Raman Study of Pressure and Temperature Effects on the Conformational Equilibrium of Diethyl Disulfide in Solutions

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The effects of pressure and temperature on the conformational equilibrium of diethyl disulfide in various organic solvents were studied by Raman spectroscopy. From the pressure and temperature dependences of the intensities of S–S and C–S stretching bands, the volume and enthalpy differences between the *trans-gauche-gauche* and *gauche-gauche-gauche* conformers in organic solvents were determined. The volume differences of -1.1–-1.3 cm^3/mol are close to the values reported previously for liquid alkanes, which are explained by the packing effect of liquid. The solvent effect on the enthalpy difference also shows that the repulsive intermolecular interaction is a crucial factor in the conformational equilibrium of diethyl disulfide in the solutions. Based on the present results, the conformational change of the disulfide bridge in protein is discussed from the viewpoint of solution chemistry.

Molecular conformational equilibrium can be affected by environmental conditions such as temperature, pressure, and solvents. Such structural flexibility plays an important role in chemical and biological properties of molecules. This is a significant reason why medium effects on conformational equilibria have been the subject of numerous experimental and theoretical studies.

A traditional treatment for the medium effects has been the dielectric continuum model, used in the classic works of Onsager and Kirkwood.[1] This theory is based on a simple model in which the solvation energy arises from the electrostatic interaction of the solute with the dielectric continuum solvent. Successful applications of this theory to the solvent effect on conformational equilibria of polar molecules have been reviewed by Abraham and Bretschneider.[2] Recent works using DFT calculations with a continuum model[3] and vibrational spectroscopies[4] have also supported the validity of the dielectric continuum model. However, this model is intrinsically useless for nonpolar solutes such as alkanes. Pratt and co-workers[5] were the first to show that the medium effects for the nonpolar molecules can be explained by the packing effect of molecules using the reference interaction site model (RISM) theory. Indeed, the volume difference between the *trans* and *gauche* conformers of butane obtained by Raman spectroscopy was qualitatively consistent with their prediction. All the high-pressure vibrational-spectroscopic works on a selection of alkanes (butanes,[6] pentane,[7] hexane,[8] heptane,[9] and hexadecane[9]) have shown similar results, that the volumes of the *gauche* conformers are smaller than those of the *trans* conformers, which is explained by the packing effect. Thus, it is acceptable for the present that medium effects on the conformational equilibria in organic solvents are explained by the following factor(s): the packing effect due to short-range repulsive interactions and/or the electrostatic effect due to long-range electrostatic interactions.

Most of the above research has targeted model molecules having internal rotational C–C bonds, such as alkanes and haloalkanes. Studies of other types of molecules are essential to obtain a comprehensive understanding of medium effects on molecular conformation. In this study, we focused on diethyl disulfide, which has a CCSSCC chain unit, as a target molecule. The S–S bond is practically rigid as the internal rotational axis so that the CSSC segment only takes the *gauche* form, while the C–S bond is flexible in the internal rotation so that the SSCC segment could take the *trans* or *gauche* form. There have been a large number of vibrational-spectroscopic studies[10,11] and normal mode and molecular orbital calculations[11] for alkyl disulfides. Assignment of the vibrational modes of diethyl disulfide has been well established.[10] In this work, we investigated the pressure and temperature effects on the conformational equilibrium of diethyl disulfide in organic solvents. Based on the accepted rule for the medium effects, the conformational thermodynamics of diethyl disulfide in organic solvents is expected to be influenced mainly by the packing effect, since the conformational changes do not induce a significant change in the dipole moment of the molecule. The packing effect is closely related to the volumetric properties of the system. Thus, pressure-perturbing experiments are an advantageous way of proving the packing contribution.

In addition, we discuss the importance of the present study from the viewpoint of protein chemistry. The diethyl disulfide skeleton (CCSSCC) is identical to the disulfide bridge in proteins. The disulfide bridge is one of the most critical factors for the structural stability of proteins. The bridges usually exist on the inside of proteins, which generally provides a nonaqueous environment. Diethyl disulfide in nonaqueous solvents would be valuable as a primary model. As the solvents, we employed the neat liquid, carbon disulfide, and methanol, which are typically nonpolar and polar solvents. Although the properties of these solvents are significantly different from each other, the

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Published on the web August 4, 2005; DOI 10.1246/bcsj.78.1411

present results of the conformational behavior showed no significant difference in terms of thermodynamics. This is consistent with the above prediction that the major contribution to the conformational thermodynamics of diethyl disulfide would be the packing effect.

Experimental

Diethyl disulfide (97% (GC)) purchased from Wako Pure Chemical Industries, Ltd., was used without further purification. Carbon disulfide and methanol purchased from Nacalai Tesque, Inc., were used after distillation. Raman spectra were recorded using a JEOL 400D spectrometer equipped with a cooled HTV-R649 photomultiplier and a photon counting system. The sample was illuminated using 90° scattering geometry by 514.5 nm radiation from an argon ion laser (NEC GLG3300) having a 200–400 mW output. The spectral resolution and scanning speed were 4.5 $\mathrm{cm^{-1}}$ and 5 $\mathrm{cm^{-1}/min}$, respectively. The reproducibility of the wavenumber was 0.6 $\mathrm{cm^{-1}}$. The high pressure cell has been described elsewhere.[12] The pressure was estimated using a Heise gauge with an accuracy of 0.5%. The temperature of the sample was controlled within an error of ±0.2 °C by circulating thermostated water around the cells. The observed spectra were fitted with Gaussian–Lorentzian product functions to determine the peak frequency and integrated intensity of each band.

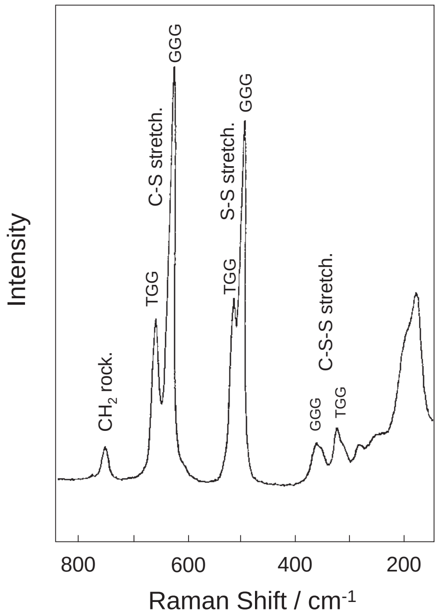

Fig. 1. Raman spectrum of liquid diethyl disulfide at 20 °C.

Results and Discussion

Raman Spectra and Peak Shifts. Figure 1 shows a typical Raman scattering from liquid diethyl disulfide, exhibiting some fundamental vibration modes. The band assignment was done on the basis of extensive works by Sugeta et al.[10] and Zhao and Krimm.[11] The present spectrum shows the existence of only *trans-gauche-gauche* (TGG) and *gauche-gauche-gauche* (GGG) conformers, indicating that these conformers are dominant in the liquid. The S–S and C–S stretching modes provide intense and well-resolved Raman bands for TGG and GGG conformers. Thus, these modes are convenient for the conformational analysis. The peak positions observed at 0.1 MPa and the mode assignments are summarized in Table 1.

Figure 2 shows Raman spectra in the region of the S–S stretching mode of (a) liquid diethyl disulfide, (b) diethyl disulfide (mole fraction: 0.5) in carbon disulfide, and (c) diethyl disulfide (mole fraction: 0.1) in methanol at 0.1 MPa and 475 MPa. These spectra show high S/N ratios and well-resolved bands. We also utilized the C–S stretching mode to monitor the conformational equilibrium. Figure 3 shows the spectra in the region of the C–S stretching mode of liquid diethyl disulfide at 0.1 MPa and 450 MPa. The assignment of the shoulder band at 620 $\mathrm{cm^{-1}}$ is not known. All the observed spectra showed high S/N ratios and well-resolved bands, fulfilling requirements for reliable line analyses. Increasing pressure influences the peak position and the intensity ratio, whose changes reflect the pressure-induced changes in the intermolecular interaction and the conformational equilibrium, respectively.

The pressure- and temperature-induced peak shifts are summarized in Table 1. It is well known that the peak shifts reflect the changes in the solute–solvent interactions: the intermolecular repulsive and attractive interactions induce blue and red shifts, respectively, of the vibrational modes. A comparison of the peak positions under various solvent conditions showed that they do not differ significantly from each other. The solvent changes do not induce significant changes in the intermolecular interaction. Although significant changes in the peak positions from pressure and temperature effects are not observed, there are a few features to describe. All the values of $\partial\nu/\partial p$ are very small and positive. The positive values for the stretching modes mean that increasing pressure increases the repulsive interaction for the stretching bond.[13] The values of $\partial\nu/\partial p$ for the neat liquid are somewhat larger than those for the solutions. Although the differences between the values for

Table 1. Raman Peak Position at 0.1 MPa and Pressure Dependence of the Peak Positions of Diethyl Disulfide in Solutions at 20 °C

Solvent[a)]	$\nu/\mathrm{cm^{-1}}$		$\partial\nu/\partial p/10^3\ \mathrm{cm^{-1}\,MPa^{-1}}$		$\partial\nu/\partial T/10^2\ \mathrm{cm^{-1}\,K^{-1}}$		Vibrational mode
(x: mole fraction)	TGG	GGG	TGG	GGG	TGG	GGG	
Neat	523.3	507.6	4.7 ± 0.4	4.4 ± 0.3	-1.4 ± 0.5	-2.4 ± 0.8	S–S stretch.
Neat	666.3	640.4	6.1 ± 0.4	5.8 ± 0.4	0.7 ± 0.6	2.0 ± 0.9	C–S stretch.
CS_2 ($x = 0.5$)	523.7	508.5	3.6 ± 0.2	3.1 ± 0.2	—	—	S–S stretch.
CS_2 ($x = 0.1$)	523.8	509.0	2.1 ± 0.3	1.8 ± 0.2	-4.6 ± 0.8	-3.7 ± 0.8	S–S stretch.
CH_3OH ($x = 0.1$)	523.8	507.6	3.3 ± 0.3	2.8 ± 0.3	-0.9 ± 0.1	-0.4 ± 0.1	S–S stretch.

a) We could not observe well-resolved C–S stretching bands in CS_2 and CH_3OH, since the Raman scattering from solvents significantly overlapped this frequency region.

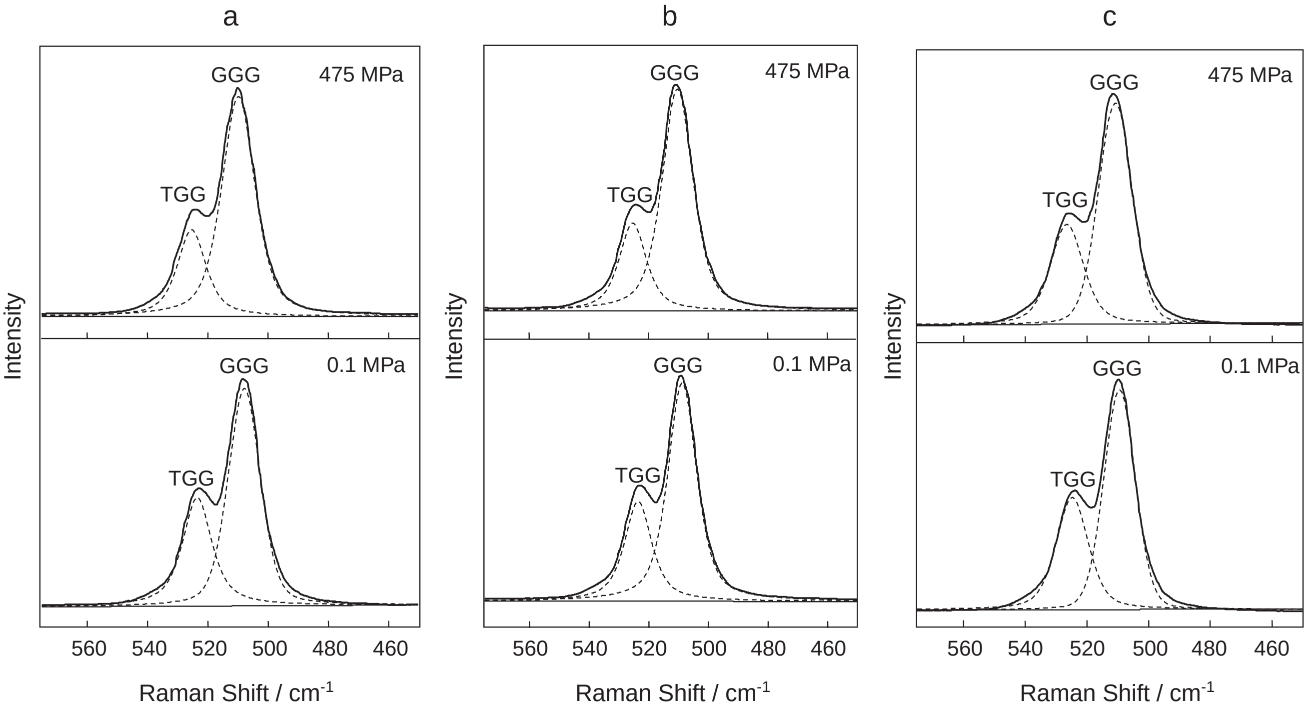

Fig. 2. Raman spectra of S–S stretching mode of (a) liquid diethyl disulfide, (b) diethyl disulfide ($x = 0.5$) in carbon disulfide, and (c) diethyl disulfide ($x = 0.1$) in methanol at 0.1 MPa and 475 MPa (20 °C).

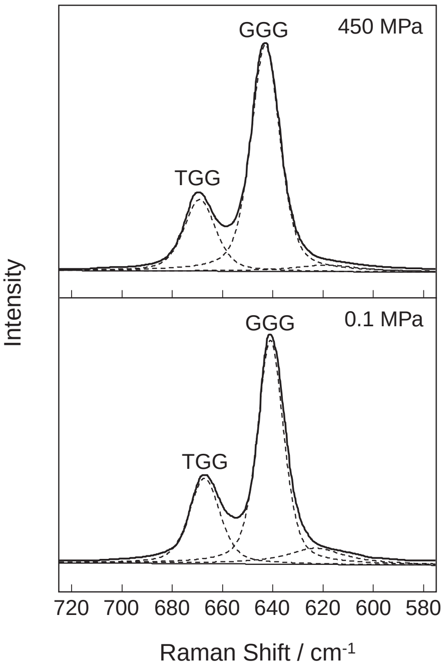

Fig. 3. Raman spectra of C–S stretching mode of liquid diethyl disulfide at 0.1 MPa and 450 MPa (20 °C).

the conformers are very slight, the values for the TGG conformer are slightly larger than those for the GGG conformer. That is, the repulsive intermolecular force increases more for the TGG conformer than the GGG conformer with increasing pressure. The bulkier conformer has a more strongly repulsive interaction with solvent molecules, which is consistent with the van der Waals picture of liquids.[14] The temperature dependence of the peak position except for the C–S stretching is consistent with the pressure dependence, assuming that the pressure dependence correlates to the density of the liquid. At present, it is not certain whether the inconsistency for the C–S stretching mode is due to the experimental error or to an insufficient model for the frequency shift.

Volume and Enthalpy Differences. The intensities of the Raman bands are essentially proportional to the populations of the corresponding conformers. The pressure dependence of the ratio of Raman intensities for conformers gives the volume difference between the conformers:

$$\Delta V = -RT\left(\frac{\partial \ln K}{\partial p}\right)_T. \tag{1}$$

Here the ratio of the integrated intensities can be substituted for the equilibrium constant K, assuming that the ratio of scattering cross sections is independent of pressure. In the same way, the enthalpy difference, ΔH, can be obtained from the temperature dependence of the ratios of the intensities:

$$\Delta H = -R\left(\frac{\partial \ln K}{\partial (1/T)}\right)_p. \tag{2}$$

Although the dependence of the scattering cross section on pressure and temperature has not been elucidated, we consider that the estimated values (ΔV, ΔH) in this work do not have a significant error, based on the following facts. If a change in pressure and temperature perturbs the normal modes, then it induces a change in the scattering cross section. The small pressure and temperature dependences of the peak frequencies in the stretching modes suggest that the equilibrated bond lengths of S–S and C–S hardly change over the measured

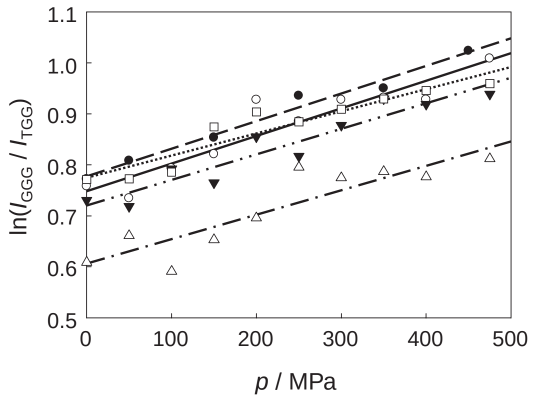

Fig. 4. Pressure dependence of the integrated intensity ratio between the TGG and GGG forms of diethyl disulfide in various solvents at 20 °C. The linear lines are obtained from the least-square fitting, with data of S–S stretching bands (○, solid line) and C–S stretching bands (■, long dashed line) of the neat liquid, and data of the S–S stretching bands in carbon disulfide ($x = 0.1$: □, short dashed line, $x = 0.5$: ▼, double short dashed line), and methanol ($x = 0.1$) (△, short and long dashed line).

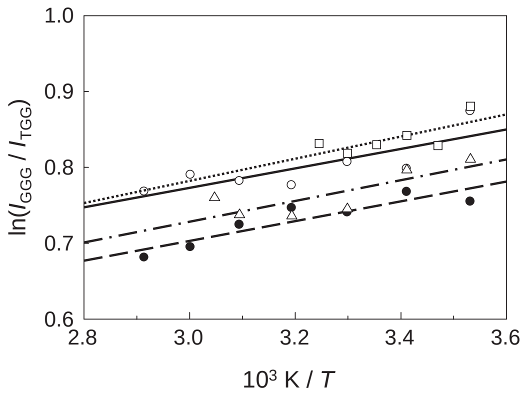

Fig. 5. Temperature dependence of the integrated intensity ratio between the TGG and GGG forms of diethyl disulfide in various solvents at 20 °C. The linear lines are obtained from the least-square fitting, with data of S–S stretching bands (○, solid line) and C–S stretching bands (■, long dashed line) of the neat liquid, and data of the S–S stretching bands in carbon disulfide ($x = 0.1$) (□, short dashed line), and methanol ($x = 0.1$) (△, short and long dashed line).

range of pressure and temperature. Furthermore, the slight differences in the frequency shifts (see Table 1) between the conformers indicate similar changes in the cross sections for the conformers. Therefore, it seems to be reasonable that the ratio of the scattering cross sections is assumed to be constant. Indeed, the obtained thermodynamic values, which we describe below, do not depend on the kind of vibrational mode utilized. This also supports the present assumption.

Figure 4 shows plots of $\ln(I_{GGG}/I_{TGG})$ versus pressure for various conditions. All the slopes of the lines are positive, indicating that increasing pressure induces an increase in the relative population of the GGG conformer. This means that a conformer with a more compact shape is more stable under high pressure. According to Eq. 1, we calculated the volume differences between the TGG and GGG conformers from the slopes of the lines. The volume differences in various solvents are summarized in Table 2. These values are approximately equal to each other, indicating no significant dependence of the volume difference on solvents and vibrational modes.

The volume differences between conformers of simple chain molecules in nonaqueous solutions have been discussed by classifying molecules into three categories: nonpolar, polar, and hydrogen bond-forming molecules. In the case of the first category, the volume difference is dominantly due to the packing effect, which is well-known as a van der Waals picture of liquids.[14] Liquid alkanes are typical examples. In the case of the second category, the volume difference is satisfactorily explained by a dielectric continuum model of solvent, as described in section 1. In the case of the third category, the change in the local solvation (hydration) structure is a crucial factor determining the volume difference.[15] In the case of diethyl disulfide, the dipole moment difference between the conformers is so small that it has a negligible contribution to the volume difference: the dipole moments of the TGG and GGG conformers are 2.31 and 2.25 D, respectively, as determined by the density functional theory calculations at the level of B3LYP/6-31G(d,p) implemented in Gaussian 03.[16] Thus, the second contribution would be negligible. Concerning the contribution of the third factor, the disulfide bond has lone pairs like the ether oxygen, which is well-known to form hydrogen bonds to OH groups. However, it is unlikely that diethyl disulfide forms a hydrogen bond to methanol because the peak positions of the S–S and C–S stretching modes in the methanol solution are not significantly different from those in the neat liquid and in CS_2. Besides, the observed volume difference of around -1.2 cm^3/mol is close to the values of -1–-1.2 cm^3/mol observed for the volume change for the single *trans* to *gauche* transformation of liquid alkanes,[6–9] which are typically classified into the first category. Hence, it is concluded that the volumetric feature of the conformation of diethyl disulfide is determined by the packing effect.

A van't Hoff plot of the equilibria between the TGG and GGG forms of diethyl disulfide in various solvents is shown in Fig. 5. We obtained the enthalpy differences between the TGG and GGG conformers from the slopes of the lines according to Eq. 2, and these values are summarized in Table 2. All the values are approximately the same. This fact is consistent with the above conclusion that the packing effect is a main contributor to the solvent effect. The packing effect on the enthalpy comes from the term $(p_{ex} + p_i)\Delta V$, where p_{ex} and p_i are the external and internal pressures, respectively. However, this term can generally be neglected.[17] In the present case, we estimated that p_i values at 20 °C for CS_2 and methanol are 350 and 360 MPa, respectively, according to $p_i = \alpha T/\beta$ using reference data[18] for α and β, which are the expansion coefficient and the isothermal compressibility, respectively. The difference of 10 MPa in p_i together with the observed ΔV gives a difference of 0.01 kJ/mol for the enthalpy, which is much smaller than the experimental errors. Thus, the solvent change does not influence the enthalpy difference of the present conformational equilibrium. Although there is no reference data for liquid diethyl disulfide, the conclusion would not be

Table 2. Volume and Enthalpy Differences between the Conformers of Diethyl Disulfide in Various Solvents at 20 °C

Solvent (x: mole fraction)	ΔV(TGG → GGG) /cm^3 mol^{-1}	ΔH(TGG → GGG) /kJ mol^{-1}	Vibrational mode
Neat	-1.3 ± 0.2	-1.1 ± 0.3	S–S stretch.
Neat	-1.3 ± 0.1	-1.1 ± 0.1	C–S stretch.
CS_2 ($x = 0.5$)	-1.2 ± 0.1	—	S–S stretch.
CS_2 ($x = 0.1$)	-1.1 ± 0.1	-1.2 ± 0.4	S–S stretch.
CH_3OH ($x = 0.1$)	-1.2 ± 0.1	-1.1 ± 0.3	S–S stretch.

affected.

Relevance to Protein Chemistry. In this work, we demonstrated that the conformational thermodynamics of diethyl disulfide in solutions is controlled by the packing effect. The skeleton of diethyl disulfide is identical to the disulfide bridge in proteins. Thus, the present results for diethyl disulfide should relate significantly to the conformational behavior of the S–S bridge portion of proteins. Finally, we make a brief remark on this point.

There have been few physicochemical studies of the conformational equilibrium of the S–S bridge of proteins. To our knowledge, there has only been one Raman study of the temperature dependence of the CSSC conformation of proteins (Bence Jones proteins), which reported that the population of TGG form increases with increasing temperature.[19] However, the S–S stretching spectra reported there were not clear, giving no conclusive results. There have been some studies of the conformational change induced by the denaturation of proteins. These reports suggest that the population of GGG form increases on heat-[20] and acid[21]-induced denaturation. A unique structure of a protein in the native state is maintained by intramolecular interactions in the protein. When the protein is unfolded, the structural stress is relaxed. Therefore, the physicochemical condition of the CCSSCC part of the unfolded protein is expected to be similar to that of the model molecule in the liquid phase.

Our remark is concerning the consistency between the observed conformational behaviors of the proteins and the present model molecule. Although the present work provided no aqueous solution data, we can predict the effect of a water solvent on the conformational equilibrium of diethyl disulfide. The present results showed that diethyl disulfide interacts repulsively with solvents including methanol. The CCSSCC part and water would also have a repulsive interaction. This is why diethyl disulfide is insoluble in water. The effect of water (hydrophobic effect) on the conformation of insoluble molecules has been an important subject and an open question.[4] There have been some reference studies on that subject. Theoretical studies[22] of butane showed that the population of the *gauche* conformer increases with a change in solvent from nonpolar solvents to water. An experimental study[4] of dichloroethane demonstrated that the *gauche* conformer becomes more favorable in water. All the studies targeting insoluble chain molecules have shown that *gauche* conformers increase with a change in solvent from nonpolar solvents to water. This rule can be applied to the case of diethyl disulfide: then the GGG conformer would become more favorable in water. Thus, this deduction is consistent with the reports[20,21] that denaturation of proteins increases the *gauche* form for the disulfide bridge conformation, considering that denaturation induces the exposure of the bridge to the solvent water.

Conclusion

From the pressure and temperature dependences of the Raman intensities of S–S and C–S stretching bands, we determined the volume and enthalpy difference between the *trans-gauche-gauche* and *gauche-gauche-gauche* conformers in organic solvents. The volume difference of -1.1–-1.3 cm^3/mol and the enthalpy difference of -1.1–-1.2 kJ/mol are explained by the packing effect of liquid. The present results show that the repulsive intermolecular interactions are dominant in the solutions, even when methanol is the solvent. This fact is useful for predicting the conformational change of the disulfide bridge of protein during denaturation.

We thank Mr. T. Yumiyama for help with the high-pressure equipment and Mr. K. Takekiyo for help with DFT calculations. This work was supported in part by Grant-in-Aid for Scientific Research (C) (No. 15550020) from Japan Society for the Promotion of Science (JSPS) and "Academic Frontier" Project from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of the Japanese Government, 2004–2008.

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