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Effect of Pressure on the Solubility of Diketopiperazine in Water[*1]

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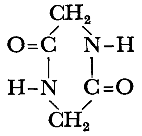
Diketopiperazine (DKP) was used as a model compound to investigate hydrogen bond interactions between the peptide bond and water under high pressure. The solubility of DKP in water was measured as a function of pressure up to about 6000 atm at 15, 25 and 35°C. It was observed that the solubility decreases with increasing pressure up to about 4500 atm, and then increases above this pressure. The solubility follows a simple exponential dependence upon pressure at least below 4500 atm. The volume change ΔV accompanying the dissolution of DKP in water is obtained from this pressure dependence of the solubility. The values of ΔV are 5.2 and 5.9 m*l*/mol at 25° and 35°C, respectively. These values coincide well with those obtained from dilatometric measurements at 1 atm. The reason why the solubility is inverted at the pressure above and below 4500 atm is ascribed to the change of water structure brought about by the compression. The effect of pressure on proteins reported in the previous paper is discussed in relation to the present solubility results.

There is evidence that the effect of pressure on proteines is inverted at the pressure above and below 3000 atm.[1)] The reason for this curious inversion may be investigated through appropriate model systems. It is well known that hydrogen bonds play a primary role in maintaining the conformation of proteins.

Diketopiperazine (DKP, ) which is a cyclic compound formed through the condensation of 2 molecules of glycine, has been used as a model compound by Gill *et al.*[2)] to investigate hydrogen bond interactions. The structure of DKP indicates the possibilities for hydrogen bonds. These possibilities are borne out in the studies of crystal structure[3)] and the infrared spectrum using polarized radiation,[4)] which show DKP molecules form linear hydrogen bonded polymers in the solid structure. The energy of hydrogen bond maintaining the DKP crystal has been found to be 7 kcal/mol-H bond from the measurements of vapor pressure of DKP by Seki *et al.*[5)]

In this report the solubility of DKP in water was measured as a function of pressure to investigate the influence of pressure on the intermolecular forces between DKP-DKP molecules and DKP-water molecules and to gain insight into the behavior of pressure on proteins. The hydrogen bonded interactions between DKP-DKP molecules are analogous to those which maintain the folded structure of proteins. The dissolution of DKP in water is assumed to be analogous to the rupture of the folded structure of proteins.

The solubility measurements of solids under hydrostatic pressure above 1000 atm are seldom found in literatures. Therefore such an experiment is worthwhile to be performed not only for DKP but also for any compound.

The volume changes ΔV that accompany the dissolution of DKP in water were measured at 1 atm by dilatometry, since such a value is a valuable quantity for predicting the initial effect of pressure upon solubility and also for investigating the interactions between DKP-DKP and DKP-water.

Experimental

Sample Preparations. DKP was prepared by the method of Dunn.[6)] The product was recrystallized twice from water. The product gave a negative ninhydrin test and was subjected to thin-layer chromato-

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1) K. Suzuki, Y. Miyosawa, M. Tsuchiya and Y. Taniguchi, *Rev. Phys. Chem. Japan*, **38**, 63 (1968).

2) S. J. Gill, J. Hutson, J. R. Clopton and M. Dowing, *J. Phys. Chem.*, **65**, 1432 (9161).

3) R. B. Corey, *J. Amer. Chem. Soc.*, **60**, 1598 (1938).

4) R. Newman and R. M. Badger, *J. Chem. Phys.*, **19**, 1147 (1951).

5) S. Seki, K. Suzuki and T. Koide, *Nippon Kagaku Zasshi*, **77**, 346 (1956).

6) M. S. Dunn, *J. Org. Chem.*, **12**, 490 (1947).

graphic analysis. The chromatography indicated no impurity.

Solubility Measurement. For the measurement of solubility of DKP a simple method of residue analysis was used. The solubility data at 25°C and 1 atm with this method was coincident with that obtained by refractive index method.[2]

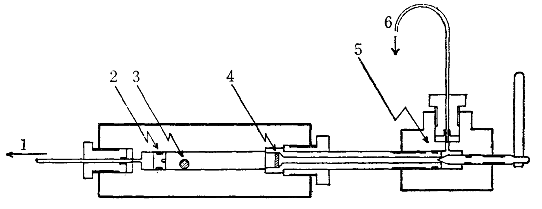

Fig. 1. High pressure equipment for measuring a solubility of solid.
1: To intensifier, 2: Piston, 3: Stainless steel ball, 4: Glass filter, 5: High pressure valve, 6: Outlet

Figure 1 shows the apparatus, which is constructed from a high pressure vessel for obtaining equilibrium solutions and high pressure valve. It is connected to an intensifier with 1/8″ diameter tubing. Glass filter is set at the outlet of the vessel to separate solid DKP from saturated solutions. High pressure measurements were performed with manganin electric-resistance gauge calibrated against Heise's precision pressure gauge of Bourdon type (50 psi scale up to 50000 psi). Paraffin oil was used as a pressure transmitting medium.

To obtain an equilibrium solution, the apparatus was shaken for about 4 hr like a seesaw in a constant temperature bath. The stainless steel ball inserted in the vessel was useful for stirring. Saturated solution was sampled out through high pressure valve by pushing the piston with oil-pump under the pressure as stable as possible. To check the apparatus and the procedures, the solubility of NaCl in water was measured at 25°C and 1000 atm. The accuracy was within ±0.2%, and the solubility data (27.06%) coincided well with that in the reference (27.07%[7]).

Dilatometry. Carlsberg type dilatometer[8] was used to measure the volume change accompanying the dissolution of DKP in water at 1 atm. Kelosine was used as the medium. It was ascertained that kerosine did not attack DKP. Before mixing, the occluded air in DKP sample was thoroughly evacuated. Thermostat was kept within ±0.003°C.

Results and Discussion

Solubility data are listed in Table 1, and are plotted in Fig. 2. These results show that solubility decreases with increasing pressure up to about 4500 atm, but this trend is inverted above this pressure.

Table 1. Solubility of DKP (wt%)

°C \ atm	1	1400	2800	4200	5600
15	1.16	0.88	0.68	0.55	0.67
25	1.66	1.25	0.93	0.68	0.82
35	2.32	1.66	1.17	0.90	1.15

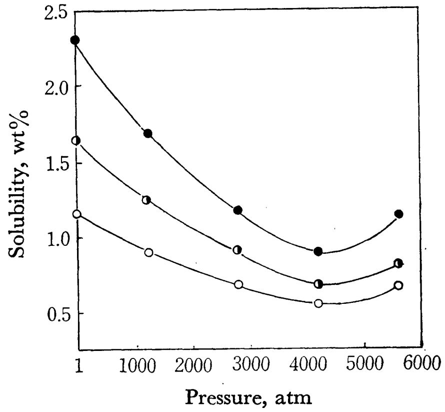

Fig. 2. Solubility of DKP *versus* pressure.
○: 15°C, ◑: 25°C, ●: 35°C

In Fig. 3 DKP solubility data at 1 atm is plotted against temperature with the data of Gill *et al.*[2] The data when plotted in logarithmic solubility (m_s, molality) *versus* $1/T$, are adequately represented by a straight line. The heat of solution ΔH is calculated from the slope using the relation

$$\left[\frac{\partial \ln m_s}{\partial(1/T)}\right]_p = \frac{-\Delta H}{R} \qquad (1)$$

The value of ΔH determined at 1 atm is 6.2 kcal/mol. This value decreases with increasing pressure, and ΔH value at 4000 atm is 3.3 kcal/mol. That is, the process of DKP dissolution in water is endothermic, and the solubility increases with rising temperature. It is noticed that the pressure effect below 4500 atm on the DKP solubility is opposite

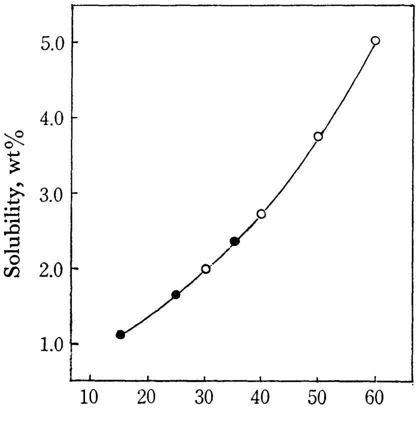

Fig. 3. Solubility of DKP *versus* temperature at 1 atm.
○: Gill *et al.*,[2] ●: This work

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[7] L. H. Adams, *J. Amer. Chem. Soc.*, **201**, 144 (1956).

[8] H. Noguchi, *Tanpakushitsu Kakusan Kōso*, **10**, 1219 (1965).

to the result of temperature.

The effect of pressure on the solubility of solid in liquid is given by the following relation, assuming the activity coefficients of both the solute and solvent are independent of the pressure,

$$\left(\frac{\partial \ln m_s}{\partial p}\right)_T \approx \frac{V'-\bar{V}}{RT} = \frac{-\Delta V}{RT} \tag{2}$$

where m_s is the molar concentration in the saturated solution, V' the molar volume of the solid, $\bar{V}$ its partial molar volume in the saturated solution and ΔV the volume change accompanying the dissolution of solid in liquid. The DKP solubility decreases with increasing pressure below 4500 atm. This means that $\bar{V}$ is larger than V', that is ΔV is positive. Above this pressure, on the other hand, ΔV changes the sign to negative. The relation between $\ln m_s$ and pressure is almost linear below 4500 atm, and the ΔV values obtained from the slope are listed in Table 2. The ΔV values above this pressure in Table 2 are calculated by Eq. (2).

TABLE 2. VOLUME CHANGE ACCOMPANYING DISSOLUTION OF DKP (m*l*/mol)

atm / °C	<4500	>4500	1*
15	+4.5	−3.9	—
25	+5.2	−5.3	+5.29
35	+5.9	−6.9	+5.88

* by dilatometric measurements

Figure 4 shows the dilatometric results measured at 1 atm. The ΔV values accompanying the dissolution of DKP at saturated concentration is obtained by extrapolating the straight line to the saturated concentration. The ΔV values thus obtained are also listed in the last column of Table 2, which coincide well with those from $\ln m_s$ *versus* pressure plot.

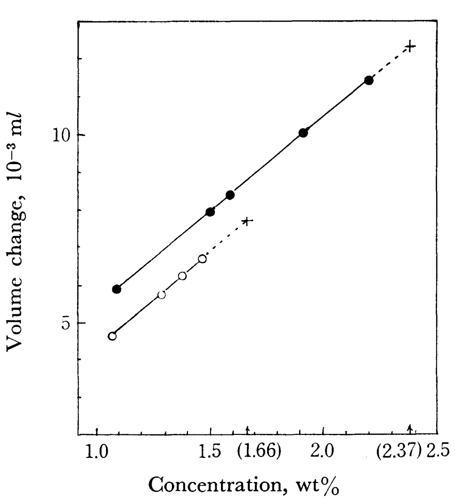
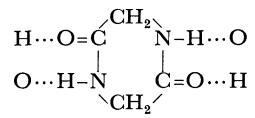


Fig. 4. Volume change accompanying dissolution of DKP.
○: 25°C, ●: 35°C, +: Saturated point

The molar volume of solid DKP is found to be 71.7 and 72.1 m*l*/mol at 25 and 35°C from our measurements of specific gravity. Therefore the partial molar volume in the saturated solution can be estimated from $\bar{V}=V'+\Delta V$. The $\bar{V}$ values thus calculated are 77.0 and 78.0 m*l*/mol at 25 and 35°C, respectively. Therefore, the difference between V' and $\bar{V}$ is only 8—9%. This suggests that the DKP dissolution in water is mono-dispersion. In view of the observed linearity of the logarithmic solubility *versus* $1/T$ plot and of the logarithmic solubility *versus* pressure below 4500 atm, mono-dispersion is ascertained over the region investigated.

The intermolecular energy maintaining the DKP crystal is estimated to be 24.83±0.51 kcal/mol from the heat of sublimation, which is the sum of the hydrogen bond energies (14.0 kcal/mol) and van der Waals energies (10.9 kcal/mol).[5] The fact that the DKP crystal dissolves in water can be understood as follows: the intermolecular forces forming the crystal are ruptured and each DKP molecule dissolves in water in hydrated state. As mentioned above, the heat of solution is 6.2 kcal/mol at 1 atm. The difference of both the values, 18.6 kcal/mol (=24.8−6.2) is to be assumed as the heat of interactions between DKP and water molecules when DKP dissolves in water. One kind of the interactions is undoubtedly the hydrogen bond between a DKP molecule and 4 molecules of water as follows:



The other kind of interaction between the hydrocarbon moiety of DKP and the surrounding water molecules is supposed when DKP molecule is transferred to water. However, the enthalpy change in the transfer of aromatic hydrocarbons to water has been found to be almost zero.[9] Therefore it will be unnecessary to consider the contribution of this interaction. As mentioned above, one molecule of DKP can interact with 4 molecules of water. Then the binding energy in a set of hydration is 4.7 kcal/mol-H bond as the mean value (18.6 kcal/mol is divided by 4). This seems to be reasonable value as the energy of hydrogen bond.

The volume change accompanying the DKP dissolution is the difference between the partial molar volume $\bar{V}$ and the molar volume of DKP crystal V' as already shown in Eq. (2). Thus, the volume change ΔV reflects the changes of intermolecular forces between DKP-DKP and DKP-

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[9] W. Kauzmann, *Advances in Protein Chem.*, **14**, 39 (1959).

water. The effect of pressure on V' is assumed to be too small to be neglected as compared to the effect on $\bar{V}$. The partial molar volume $\bar{V}$ is influenced by the hydrogen bonds between DKP and water, that is, NH···O and CO···H, and the interaction between hydrocarbon moiety of DKP and water such as in "icebergs".[10] The positive value of ΔV below 4500 atm means that $\bar{V}$ is larger than V', and the negative value of ΔV above 4500 atm means that V' is larger than $\bar{V}$. This inversion must be ascribed mainly to the decrease of $\bar{V}$ above 4500 atm. This volume contraction might be probably deduced, because the interactions between DKP and water molecules become very strong above 4500 atm owing to the change of water structure brought about by the compression.

It is very interesting to compare the pressure effect on the DKP solubility with the influence of pressure on proteins. The higher pressure above 3000 atm causes the denaturation of proteins, while the lower pressure below 3000 atm retards the heat denaturation of proteins and accelerates the recovery of proteins once denatured by heat and pressure.[1] The denaturation phenomenon in which the folded structure of proteins changes into the unfolded structure is analogous to the dissolution of DKP crystal in water. That is, the folded protein corresponds to the DKP crystal, and the unfolded protein corresponds to the dissolved DKP in the hydrated state. If V' is the molar volume of the folded protein, and $\bar{V}$ is that of the unfolded protein, the sign of the volume change ΔV $(=\bar{V}-V')$ defines the direction of the process: folded protein (native) $\rightleftarrows$unfolded protein (denatured). It is plausibly supposed that at the higher pressure above 3000 atm where the proteins are denatured, ΔV becomes negative, while at the lower pressure below 3000 atm where the denatured proteins are recovered, ΔV becomes positive as in the case of the DKP dissolution in water. This inversion must be ascribed mainly to the large decrease of $\bar{V}$ above 3000 atm, because the interaction between protein and water molecules becomes so strong due to the change of water structure above this pressure that the interaction maintaining the folded structure of protein is ruptured. The discrepancy in the pressure (4500 atm in DKP and 3000 atm in proteins) where the inversion occurs is not fatal, because at least in proteins this inversion pressure is considerably influenced by the change of the state of medium such as pH, buffer, ionic strength and so on as well as the difference of proteins.

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[10] H. S. Frank and M. W. Evans, *J. Chem. Phys.*, **13**, 507 (1945).