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Physico-chemical Approach to Biopharmaceutical Phenomena. II.[1] Hydrophobic Hydration of Tryptophan in Aqueous Solution[2]

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In order to discuss the entropy increase on the adsorption process of tryptophan by carbon black from aqueous solution[1], the water–tryptphan interaction was investigated on the basis of solubility properties of tryptophan in water and in aqueous urea solution.

Calculating the thermodynamic functions of the dissolution of tryptophan in water, the entropy change was negative, meaning that the water structure changed to more ordered one by dissolving tryptophan in water, that is, an iceberg might be formed in the neighborhood of hydrophobic moiety of tryptophan, *i.e.*, indole group.

Calculating the thermodynamic functions of the transfer of tryptophan from aqueous solution to aqueous urea solution, the entropy change increased with the concentration of urea and the enthalpy change was negligible, as were contrary to those of a general complex formation. It was reasonable that the solubility of tryptophan increased with the entropy increase through the break-down of iceberg with the addition of urea in aqueous solution of tryptophan, *i.e.*, through the decrease of hydrophobic bonding.

Finally it was concluded that the entropy increase overcame the enthalpy increase in the adsorption of tryptophan by carbon black from aqueous solution. In other words, the adsorption[1] was forced with the entropy increase caused by the change of water structure.

The solvent effect is characteristic of the adosprtion from solution, generally making the phenomena more complicated than gas adsorption. Therefore, data regarding the solvent effect may give a useful information for understanding the mechanism of the adsorption process, as Corkill[4] and Kipling[5] suggested. It is considered that the solvent effect is based on (1) solvent–adsorbent interaction (*i.e.*, adsorption of solvent) and (2) solvent–adsorbate interaction, though very few investigations have been done on the latter, *e.g.*, on the effect of solubility.[5]

In the previous paper,[1] the thermodynamic functions obtained showed that the entropy effect was predominant on the adsorption of tryptophan by carbon black from aqueous solution. It was suggested for this result that the adsorption might be forced with the entropy increase through the change of water structure caused by break-down of the iceberg enclosing the tryptophan molecule in solution. In other words, this adsorption might be influenced by the solvent–adsorbate interaction, *i.e.*, the hydrophobic hydration of tryptophan. The solvent–adsorbent interaction, *i.e.*, the adsorption of water by carbon black, was negligible because tryptophan was considered to be adsorbed preferentially.

The present study was attempted to investigate the water–tryptophan intereaction on the basis of solubility properties of tryptophan in water and in aqueous urea solution, as

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[1] Part I: H. Nogami, T. Nagai, E. Fukuoka, H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **16**, 2248 (1968). This paper forms Part XXII of "Studies on Powdered Preparations."

[2] A part of this work was presented at the 87th Annual Meeting of Pharmaceutical Society of Japan, Kyoto, April 1967, being taken in part from the thesis of Hiroshi Uchida for the degree of Doctor of Pharmaceutical Sciences, University of Tokyo, 1968.

[3] Location: *Hongo, Tokyo*; *a*) Fellow from *Chemical Laboratory, Kyorin Pharmaceutical Co., Ltd., Ukima-machi, Kita-ku, Tokyo.*

[4] J.M. Corkill, J.F. Goodman, and J.R. Tate, *Trans. Faraday Soc.*, **61**, 979 (1965).

[5] J.J. Kipling and P.V. Shooter, *J. Colloid Interface Sci.*, **21**, 595 (1966).

was helpful to discuss the entropy increase on the adsorption process described above. Urea, which was known to disrupt the water structure,[6] was a suitable material to ascertain the hydrophobic hydration of tryptophan in aqueous solution.

Experimental

Materials——DL-Tryptophan and urea of the puerst reagent grade were obtained commercially.

Procedure for the Determination of Solubility of Tryptophan——Excess of tryptophan was added in 25 ml of water or aqueous urea solution, being shaken in a constant temperature incubator (Taiyo M-1) for 24 hr,[7] and the solution was taken out with a cotton-filter[8] attached pipette. The concentration of tryptophan was determined at 280 mμ according to UV absorption method after diluting with distilled water, using a Hitachi Perkin-Elmer UV-VS spectrophotometer. For the investigation of pH dependence of solubility, tryptophan was dissolved in a mixture of 1/10 N HCl and 1/10 N KOH,[9] the equilibrium pH being determined with a Hitachi-Horiba Model P pH meter.

Results and Discussion

Solubility Properties of Tryptophan in Water

Fig. 1 shows the pH dependence of solubility of tryptophan, from which it was ascertained that pK_1=2.38 and pK_2=9.39. In the same way as in the adsorption of tryptophan by carbon black,[1] the solubility did not depend on pH in the region of pH 3 to 9. Cation and anion of tryptophan should increase in strong acidic and in strong alkaline pH region, respectively. Therefore, if such ion might be adsorbed, the amount of tryptophan adsorbed should increase with acidity or alkalinity. However, the result obtained in the previous paper[1] was on the contrary. Accordingly, this fact seemed to confirm that the adsorption of tryptophan by carbon black was based on the hydrophobic bonding, as was discussed in the previous paper.[1] Moreover, it was established that the tryptophan molecule in aqueous solution kept its hydrophobic property in a wide pH range. The experiments for the following discussions were carried out in an aqueous solution of tryptophan contianing no buffer.

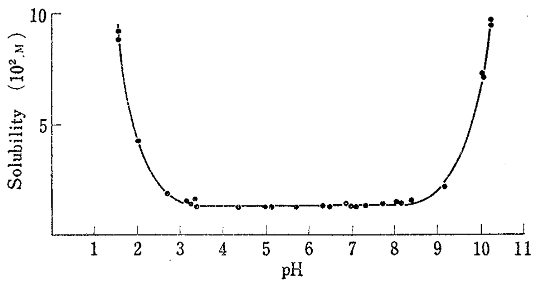

Fig. 1. pH Profile of Solubility of DL-Tryptophan in Water at 30°

Next, the thermodynamic functions of dissolution of tryptophan in water were investigated. Considering that the solubility is of the order of 10^{-2}M, the solution can be treated as a dilute one, and thus the mole fraction of tryptophan, X_T, is nearly equal to the solubility in mole unit, S_T. Then, the following equations are derived.

$$\Delta F = -RT\ln X_T = -RT\ln S_T \tag{1}$$

$$\Delta H = d(\Delta F/T)/d(1/T) \tag{2}$$

$$\Delta S = (\Delta H - \Delta F)/T \tag{3}$$

where ΔF, ΔH, and ΔS are the free energy change, the enthalpy change, and the entropy change, respectively, at temperature T. Fig. 2 shows the slope of the plot of log S_T against $1/T$ was supposed to be constant in the present temperature range, the value, ΔH, being calculated as 4.51 kcal/mole according to equations (1) and (2). The thermodynamic functions

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6) J.A. Rupley, *J. Phys. Chem.*, **68**, 2002 (1964).
7) This was satisfactorily long to attain to equilibrium.
8) The adsorption of solute by the filter was preliminarily examined to be negligible.
9) This was to keep the ionic strength 0.1.

obtained are shown in Table I. ΔF and ΔH were positive, while ΔS was negative. Generally, a solubility of organic substance increases with temperature, that is, the dissolution process is endothermic. Tryptophan was the same with respect to this point. On the other hand, it is general that the entropy increases through a dissolution process as one of the dispersion processes. However, the entropy decreased in the dissolution of tryptophan in water. Here, the dissolution process is considered to be devided into two steps, *i.e.*, fusion and mixing. The heat of fusion of tryptophan seems positive and large, since the aggregation energy of tryptophan crystal is expected to be large because of the high decomposition point, and of the ionic structure observed from IR absorption spectrum. The mixing step may be made of the dispersion and hydration of tryptophan, and the heat of hydration generally is exothermic. Regarding the dissolution process of tryptophan, it was considered that the heat of fusion overcame the heat of hydration, giving the positive value of ΔH. On the other hand, it was considered that the entropy decrease of hydration overcame the entropy increase of fusion and dispersion, giving the negative value of ΔS.

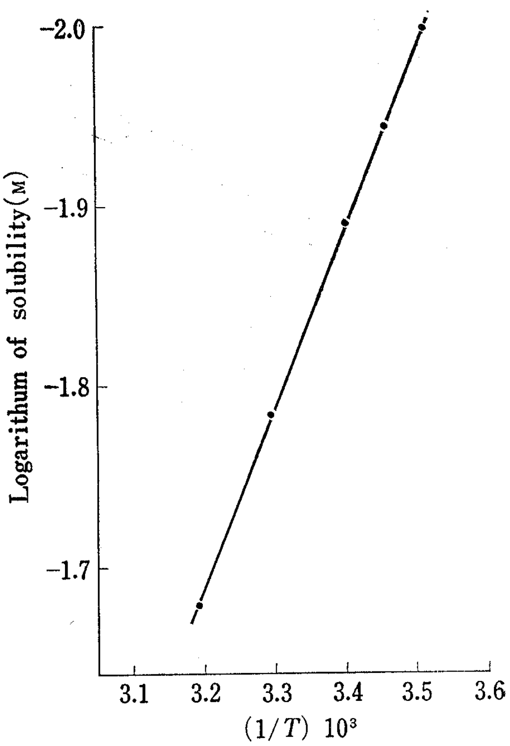

Fig. 2. Relationship between Solubility of DL-Tryptophan and Temperature

TABLE I. Solubility of DL-Tryptophan in Water and Thermodynamic Functions of Dissolution

Temperature (°C)	Solubility (10^2M)	ΔF (kcal/mole)	ΔH (kcal/mole)	ΔS (e.u.)
20	1.02	5.02	4.51	-1.73
25	1.14	5.03	4.51	-1.74
30	1.25	5.04	4.51	-1.75
40	1.64	5.06	4.51	-1.76
50	2.15	5.04	4.51	-1.70

As a result, the negative value of ΔS obtained above might mean that the water structure changed to more ordered one by dissolving tryptophan in water. Such a change of water structure has been reported on the dissolution of hydrocarbons,[10] large hydrophobic molecules[11] and others in water, accompanying the entropy increase. This kind of hydration is some different from usual hydration phenomena and is generalized as "hydrophobic hydration." The term "iceberg" is given to the ice-like structure of water formed by this hydration in the neighborhood of the hydrophobic group. It might be possible that an iceberg was formed in the neighborhood of hydrophobic moiety of tryptophan, *i.e.*, indole group.

Solubility Properties of Tryptophan in Aqueous Urea Solution

Solubility of tryptophan was determined in aqueous urea solution of various concentrations at different temperatures, as shown in Fig. 3. Since urea disrupts the water structure,[6]

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[10] W.F. Claussen and M.F. Polglase, *J. Am. Chem. Soc.*, **74**, 4817 (1952).
[11] Y. Kuroki and A. Katayama, *Hyomen*, **3**, 233 (1965).

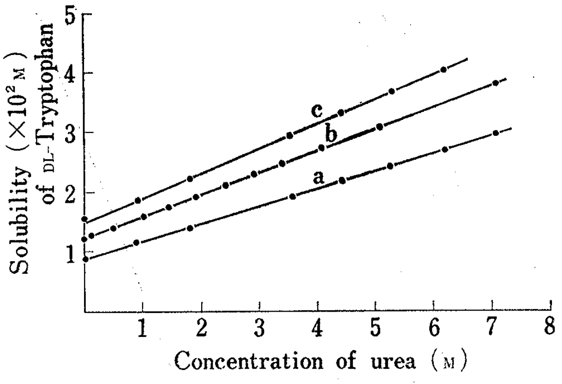

Fig. 3. Effect of Urea on Solubility of DL-Tryptophan

a. 21° b. 30° c. 40°

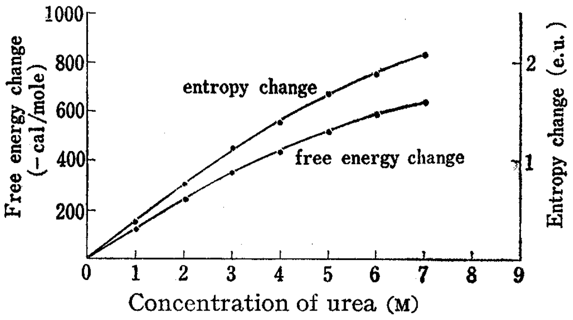

Fig. 4. Free Energy and Entropy Changes of Transfer of DL-Tryptophan from Aqueous Solution to Aqueous Urea Solution at 30°

it is expected that the entropy of the system increases with the addition of urea. Therefore, the thermodynamic functions of the transfer of tryptophan from aqueous solution to aqueous urea solution were calculated in order to discuss whether an iceberg was formed in aqueous solution of tryptophan. Neglecting the change of activity coefficient, the free energy change, ΔF_t, of the transfer is expressed as

$$\begin{aligned}\Delta F_t &= RT\ln(X_{Tw}/X_{Tu})\\ &= RT\ln(S_{Tw}/S_{Tu}) \qquad (4)\end{aligned}$$

where X_{Tw} and X_{Tu} are the mole fractions of tryptophan in water and in aqueous urea solution, respectively, and S_{Tw} and S_{Tu} are the solubilities of tryptophan in water and in aqueous urea solution, respectively. The enthalpy change, ΔH_t, and the entropy change, ΔS_t, are calcualted in the same way as in equations (2) and (3).

Table II. Thermodynamic Functions of Transfer of DL-Tryptophan from Aqueous Solution to Aqueous Urea Solution at 30°

Concn. of urea (M)	ΔF_t (kcal/mole)	ΔH_t (kcal/mole)	ΔS_t (e.u.)
1	-0.12	0	+0.39
2	-0.24	0	+0.75
3	-0.35	0	+1.12
4	-0.43	0	+1.39
5	-0.52	0	+1.68
6	-0.58	0	+1.88
7	-0.64	0	+2.08

The results obtained are shown in Table II. The entropy change, ΔS_t, increased with the concentration of urea, as shown in Fig. 4.

The enthalpy change, ΔH_t, was negligible. It is known well that a complex is often formed between urea and organic substance, *e.g.*, benzoic and salicylic acid[12] accompanying the increase of solubility. In such a complex formation generally, enthalpy decreases in the amount of several kcal/mole, and entropy also decreases. Since the results obtained above were on the contrary, it was considered that any complex was not formed between

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[12] S. Bolton, *J. Pharm. Sci.*, **52**, 1071 (1963).

tryptophan and urea. This was certified also from the UV absorption spectrum. Moreover, the stability constants, calculated on the assumptions of the formation of one to one complex because of the linear increase of solubility as shown in Fig. 3, were 0.36 at 21°, 0.30 at 30° and 0.26 at 40°, being remarkably small compared with those of usual cases.[13]

As a result, it was reasonable that the solubility of tryptophan increased with the entropy increase through the break-down of iceberg with the addition of urea in aqueous solution of tryptophan *i.e.*, through the decrease of hydrophobic bonding. Recently, Feldman and Gibardi gave a new explanation based on the decrease of hydrophobic bonding[14] to the increase of solubility of benzoic or salicylic acid in aqueous urea solution, which had been explained on the basis of complex formation.[15] Nozaki and Tanford reported the free energy change of the transfer of amino acids from aqueous solution to aqueous urea solution increased with the size of non-polar group, as was related with the necessity of hydrophobic bonding for the stability of proteins in aqueous solution.[16]

Discussion of the Adsorption of Tryptophan by Carbon Black from Aqueous Solution

As was discussed above, it is considered that tryptophan molecule is enclosed in an iceberg in water. Therefore, if carbon black is in existence, tryptophan in solution may tend to come in contact with carbon black by adopting an orientation of hydrophobic moiety to the carbon black surface so as to make an decrease in the contact area between tryptophan and water. Through this process the entropy is expected to increase as shown schematically

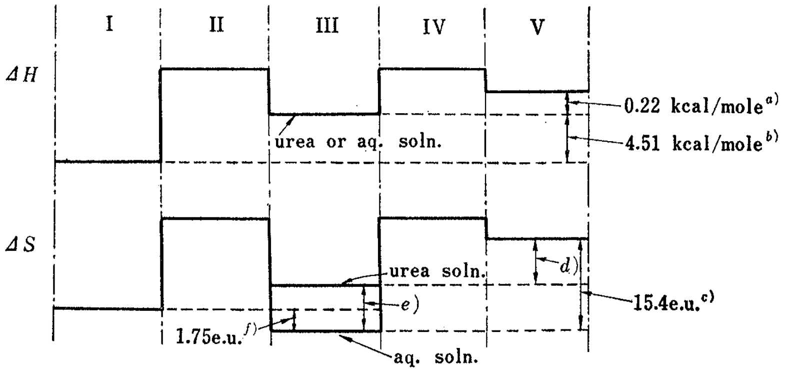

Fig. 5. Schematic Diagram of Dissolution and Adsorption Processes of Tryptophan at 30°

I : crystalline state
II : dispersed state } dissolution processs
III: hydrated state (iceberg formation) } dissolution processs
IV: dehydrated state (iceberg destruction) } adsorption process
V : adsorbed state } adsorption process

a) ΔH of adsorption from aqueous or aqueous urea solution. See Table IV in the previous paper.[1]
b) ΔH of dissolution in water or aqueous urea solution. See Table I.
c) ΔS of adsorption from aqueous solution. See Table IV in the previous paper.[1]
d) ΔS of adsorption from aqueous urea solution, depending on the concentration of urea. See Table I in the following paper (Part III: H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **16**, 2271 (1968).
e) ΔS of transfer from aqueous solution to aqueous urea solution, depending on the concentration of urea. See Table II.
f) ΔS of dissolution in water. See Table I.

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13) T. Higuchi and K.A. Connors, *Advan. Anal. Chem. Instr.*, 4, 117 (1965).
14) S. Feldman and M. Gibardi, *J. Pharm. Sci.*, **56**, 370 (1967).
15) D.M. Altwein, J.N. Delgado, and F.P. Cosgrove, *J. Pharm. Sci.*, **54**, 603 (1965).
16) Y. Nozaki and C. Tanford, *J. Biol. Chem.*, **238**, 4074 (1963).

in Fig. 5 in which the data are from both this and the previous paper.[1] The adsorption process is considered to be devided into two steps: (1) the dehydration of molecule accompanying the destruction of iceberg and (2) the interaction of molecule with the surface of adsorbent accompanying the hydration and the iceberg formation in part. Thus, it is possible that the enthalpy increases, as shown in Fig. 5. However, the entropy increase overcame the enthalpy increase in the adsorption of tryptophan by carbon black from aqueous solution. Conclusively, the adsorption was forced with the entropy increase caused by the change of water structure.