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Physico-chemical Approach to Biopharmaceutical Phenomena. III.[1] Hydrophobic Bonding in the Adsorption of Tryptophan by Carbon Black from Aqueous Solution[2]

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The results obtained as follows were satisfactory enough to demonstrate the hydrophobic bonding mechanism of the adsorption of tryptophan by carbon black from aqueous solution and also the causative role of the hydrophobic moiety of molecule.

Calculating the thermodynamic functions of the adsorption by carbon black from aqueous urea solution, the absoulte values of both free energy change and entropy change decreased, as was considered to be originated from the change of property of tryptophan in solution with the addition of urea. That is, the iceberg enclosing the tryptophan molecule in solution was broken down with the addition of urea.

Glycine and leucine were negligibly little adsorbed by carbon black from aqueous solution. Phenylalanine was less adsorbed than tryptophan. These results followed the order of hydrophobicity of molecule. The temperature dependence of the adsorption of phenylalanine was usual one, that is, an energy-predominant adsorption took place contrary to the case of tryptophan, while leucylglycylphenylalanine gave the same type of temperature dependence as tryptophan. This means the entropy change of adsorption of phenylalanine was too small to overcome the enthalpy effect.

In the previous papers,[1,4] the adsorption of tryptophan by carbon black from aqueous solution was considered to 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. It was described that urea disrupted such iceberg in solution.[1] Accordingly, if urea is added in the tryptophan/carbon black/water system, the entropy increase of adsorption, *i.e.*, the driving force for the adsorption of tryptophan, is expected to decrease, resulting in a decrease of the adsorption. Therefore, an experimental demonstration of the above consideration may correspond to a certifying of the hydrophobic bonding mechanism in the adsorption of tryptophan by carbon black from aqueous solution.

The present study was carried out from the above point of view, determining the amount of tryptophan adsorbed by carbon black from aqueous urea solution. Additionally, the adsorptions of glycine, leucine, phenylalanine and leucylglycylphenylalanine were examined in order to ascertain that the hydrophobic moiety of the adsorbate molecule played an important role in the adsorption of amino acid by carbon black from aqueous solution.

Experimental

Materials——Carbon black used was the same as that in the previous paper.[4] DL-Tryptophan, glycine, DL-leucine, DL-phenylalanine, DL-leucylglycyl-DL-phenylalanine, and urea of the purest reagent grade were obtained commercially.

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[1] Part II: H. Nogami, T. Nagai, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **16**, 2265 (1968). This paper forms Part XXIII of "Studies of 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.

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[4] H. Nogami, T. Nagai, E. Fukuoka, and H. Uchida, *Chem. Pharm. Bull.* (Tokyo), **16**, 2256 (1968).

Procedure for Determination of the Amount Adsorbed by Batch Method——The adsorption procedure was carried out in the same as described in the previous paper.[4]

Analytical Methods——The concentrations were determined as follows: a) Tryptophan[5]: in the same way as in the previous paper[1,4]; b) Glycine and leucine: according to Yemm and Cocking's method,[6] usnig ninhydrin as the color reagent; c) Phenylalanine: at 257.5 mμ according to UV absorption method; d) Leucylglycylphenylalanine: at 257.5 mμ according to UV absorption method, using a long-path cell; e) Urea[5]: at 430 mμ colorimetrically according to Brown's method,[7] using *p*-dimethylaminobenzyl aldehyde as the color reagent.

Results and Discussion

Adsorption of Tryptophan by Carbon Black from Aqueous Urea Solution

The adsorption isotherms obtained in various concentrations of urea were well described with Langmuir equation, as shown in Fig. 1. The adsorption of tryptophan decreased with the amount of urea added in the system. Actually, some amount of urea was adsorbed by carbon black from the aqueous solution containing no tryptophan, but tryptophan was preferentially adsorbed from the solution containing both tryptophan and urea, the amount of urea adsorbed being negligible. Therefore, the decrease of adsorption of tryptophan with the addition of urea should be due to reasons other than the adsorption of urea.

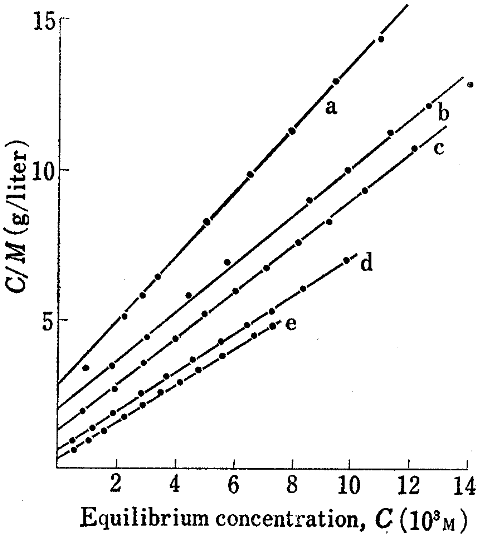

Fig. 1. Langmuir Plot of Adsorption of DL-Tryptophan by Carbon Black from Aqueous Urea Solution at 30°

a: 7.07 M d: 1.01 M
b: 5.03 M e: 0.107 M
c: 3.05 M

In the same way as in the previous paper,[4] the thermodynamic functions were calculated from the Langmuir constants *a* and *b*, *i.e.*, the amount adsorbed when the entire surface is covered by a monolayer and the equilibrium constant of the adsorption process, respectively. Since the enthalpy change of the transfer of tryptophan from aqueous solution to aqueous urea solution had been zero,[1] the enthalpy change of adsorption of tryptophan

Table I. Langmuir Constants and Thermodynamic Functions of Adsorption of DL-Tryptophan from Aqueous Urea Solution by Carbon Black at 30°

Urea concentration M	*a*-Value[a] $\frac{10^3\ \text{mole}}{\text{g}}$	*b*-Value[a] $\frac{10^{-3}\ \text{liter}}{\text{mole}}$	ΔF $\frac{\text{kcal}}{\text{mole}}$	ΔH $\frac{\text{kcal}}{\text{mole}}$	ΔS e.u.	ΔS_t[b] e.u.	ΔS_s[c] e.u.
0	1.63	1.70	-4.45	0.22	15.4	0	15.4
0.11	1.60	1.70	-4.45	—	15.4	0.05	15.5
1.01	1.54	1.08	-4.18	—	14.5	0.38	14.9
3.05	1.28	0.615	-3.87	—	13.5	1.15	14.7
5.03	1.18	0.472	-3.70	—	12.9	1.68	14.6
7.07	0.92	0.397	-3.57	—	12.5	2.08	14.6

a) from Langmuir equation
b) entropy change of the transfer of tryptophan from aqueous solution to aqueous urea solution from the previous paper[1]
c) $\Delta S_s = \Delta S + \Delta S_t$

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5) Tryptophan and urea did not interfere with each other in the respective determinations.
6) E.W. Yemm and E.C. Cocking, *Analyst*, **80**, 209 (1955).
7) H.H. Brown, *Anal. Chem.*, **31**, 1844 (1959).

from aqueous urea solution was considered to be equal to that from aqueous solution, being 0.22 kcal/mole. Thus, the results were obtained as shown in Table I.

The absolute values of both the free energy change, ΔF, and the entropy change, ΔS, decreased with the increase of urea concentration. The interaction of tryptophan with the carbon black surface was considered not to differ with the existence of urea, because the adsorption of urea was not observed under the experimental conditions. Therefore, the changes of thermodynamic functions might be oirginated from the change of property of tryptophan in solution with the addition of urea, as was also confirmed by the following consideration.

As described in the previous paper,[1] the entropy of tryptophan in aqueous urea solution was higher than that in aqueous solution. In this connection, summing the entropy changes of the adsorption of tryptophan by carbon black from aqueous urea solution, ΔS, and of the transfer of tryptophan from aqueous solution to aqueous urea solution, ΔS_t,[1] it was almost constant irrespective of the urea concentration as shown in Table I. In other words, it was possible that the iceberg enclosing the tryptophan molecule in solution was broken down with the addition of urea, resulting in an decrease in "the entropy increase," *i.e.*, the driving force in the adsorption of tryptophan by carbon black from aqueous solution, and thus the adsorption decreased. Finally, it was established that the present adsorption phenomenon was based on the hydrophobic bonding mechanism.

Adsorption of Glycine, Leucine, Phenylalanine, and Leucylglycylphenylalanine by Carbon Black from Aqueous Solution

Amounts of both glycine and leucine adsorbed were negligibly small. Although phenylalanine was adsorbed pretty much by carbon black, it was less adsorbed compared with tryptophan. These facts show that the adsorption increases with the size of hydrophobic moiety. Tanford, *et al.*[8] reported that the decreasing order of free energy change of the transfer of amino acids from aqueous solution to aqueous urea solution was as follows: tryptophan> phenylalanine> leucine, being the same as the above result. This seems to be of the order of hydrophobicity of molecule.

On the adsorption of phenylalanine as shown in Fig. 2, a negative adsorption appeared in a low concentration of phenylalanine. According to the method applied in this study, a result of negative adsorption is given in such a case that the solvent (water) was adsorbed more than the adsorbate. Since carbon black used seemed to have some polar part on the surface,[4] water might be adsorbed on that part, resulting in a negative adsorption. If the solute has a high adsorbability like tryptophan, it may be adsorbed preferentially on the carbon black, hindering the water from attaching to the polar part.[4] Thus, the adsorption of water can be negligible. However, since phenylalanine has not so high adsorbability, water is expected to be adsorbed on the polar part, especially, in the low concentration of phenylalanine, as might be the reason of the negative adsorption of phenylalanine described above. In this connection, the intrinsic amount of phenylalanine adsorbed was considered to be larger than the plot shown in Fig. 2.

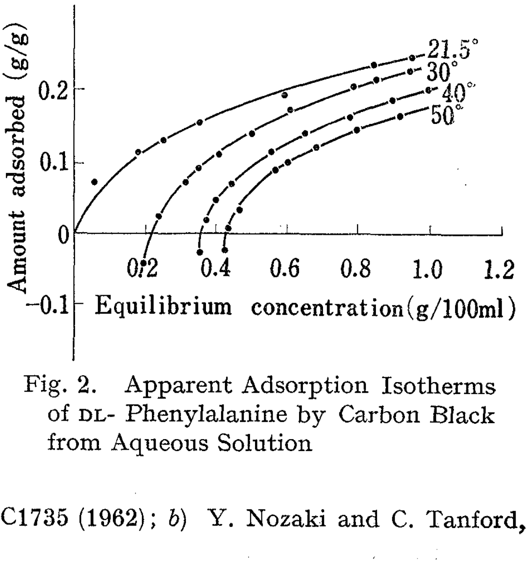

Fig. 2. Apparent Adsorption Isotherms of DL- Phenylalanine by Carbon Black from Aqueous Solution

The temperature dependence of adsorption of phenylalanine was usual one, as shown in

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[8] *a*) P.L. Whitney and C. Tanford, *J. Biol. Chem.*, **237**, PC1735 (1962); *b*) Y. Nozaki and C. Tanford, *J. Biol. Chem.*, **238**, 4074 (1963).

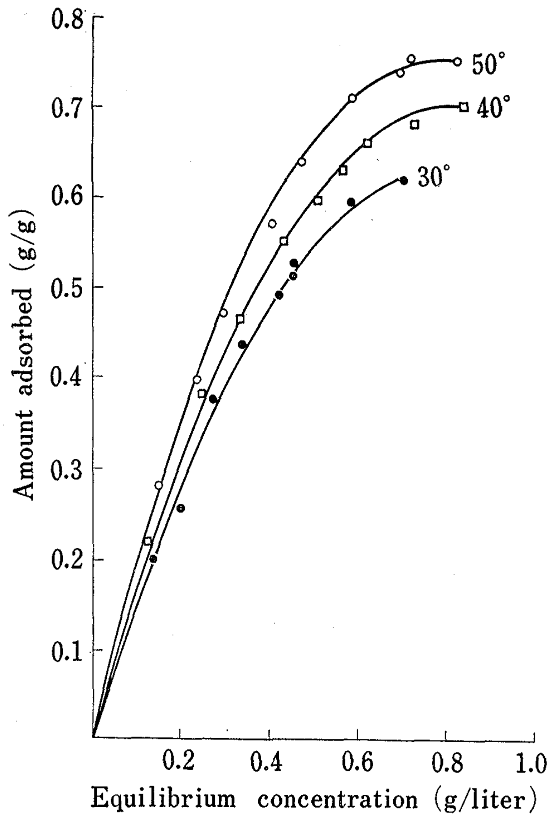

Fig. 3. Adsorption Isotherms of DL-Leucyl glycyl-DL-phenylalanine by Carbon Black from Aqueous Solution

Fig. 2, that is, an energy-predominant adsorption took place contrary to the case of tryptophan. This means that the effect of the interaction of phenylalanine with the surface, *i.e.*, exothermic process, overcame that of the break-down of iceberg, *i.e.*, endothermic process, and also means that the hydrophobicity of tryptophan was greater than that of phenylalanine and thus the entropy change caused by break-down of the iceberg enclosing tryptophan was greater.

The result obtained for leucylglycylphenylalanine is shown in Fig. 3. The adsorption increased with temperature in the same way as in the case of tryptophan.⁴⁾ Accordingly, it was possible that the hydrophobic bonding played an important role as the driving force also for the adsorption of this peptide which has hydrophobic property, supporting the result obtained in the case of tryptophan. Since hydrophobic bonding is expected to increase with the size of hydrophobic moiety, the result obtained above seems quite reasonable, corresponding to the fact that the uptake of amino acids from intestinal segment rised with the hydrophobicity of molecule.⁹⁾

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9) L.R. Finch and F.J.R. Hird, *Biochim. Biophys. Acta*, 43, 278 (1960).