Thermodynamic Aspects of Hydrophobicity and the Blood−Brain Barrier Permeability Studied with a Gel Filtration Chromatography

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It has been said that the selective permeability across the blood−brain barrier depends on several physicochemical properties of drugs such as hydrophobicity, molecular weight, and hydrogen-bonding potential. In order to investigate quantitatively the relationship between the blood−brain barrier permeability and the hydrophobicity of drugs, we have measured the micelle/water partition properties (P_{mic}) by the MLC (micellar liquid chromatography) method using a gel filtration version and compared with the blood−brain barrier permeability. The thermodynamic aspects of partition were derived by separating the micelle/water partition coefficient (log P_{mic}) into the enthalpy term P_H and the entropy term P_S. It was found that the P_H shows a good correlation to the permeability, although log P_{mic} fails to do so. The result means that P_H, which is easily obtained from the *in vitro* experiment, can be used as an excellent standard in discussing the transport phenomena through the blood−brain barrier.

Introduction

The entry of many drugs from blood to brain is restricted by a barrier separating the brain interstitial space from blood.[1] This is called the blood−brain barrier (BBB) and is constituted of characteristic tissue structures such as tightly joined brain capillary endothelial cells, few pinocytic vesicles, and the absence of transport pores.

The drug permeation through the blood−brain barrier has long been believed to depend on lipid solubility and molecular weight.[2] Under this idea it was reported that the logarithm of octanol/water partition coefficient (log *P*) is directly related to the BBB permeability.[3] In another report, log $P/M^{1/2}$, the logarithm of octanol/water partition coefficient divided by the square root of molecular weight (*M*), showed a good relationship to the BBB permeability.[4] Peptide lipophilicity was also reported to be of major importance in determining penetration across the BBB.[5] However some exceptions were found for one class of peptides, suggesting that a few unidentified factors might be important in the BBB penetration. Quite recently hydrogen-bonding potential was proposed as a main factor of the peptide permeability across the BBB.[6] In this case octanol/water partition coefficients did not correlate with the permeability, whereas heptane/ethylene glycol partition coefficients or differences in partition coefficients between octanol/water and isooctane/water systems succeeded in doing so. All of these studies show that some physicochemical properties of drug relate to the BBB permeability, but hitherto the decisive universe scale has not been reached. In the present study, the authors attempted to find any new relations between the physicochemical properties and the BBB permeability with the aid of thermodynamic consideration of hydrophobicity. The thermodynamic parameters used are the hydrophobic enthalpy constant (P_H) and the hydrophobic entropy constant (P_S) proposed by the authors.[7] These novel parameters are determined experimentally by means of gel filtration chromatography which utilizes micelle solution as mobile phase. The authors have proposed the micelle chromatography as a versatile experimental method of estimating molecular hydrophobicity.[8]

Micelle chromatography can give the micelle/water partition coefficient (P_{mic}) without necessity of high purity and large amount of samples. Although alternative hydrophobicity scales were proposed[9] by giving attention to only capacity factor (k') in the chromatographic measurement, k' includes P_{mic} as well as P_{sta} (stationary phase/water partition coefficient) as described in eq 1 below. Hence, k' is not a unique descriptor of the partition coefficient P_{mic}. The authors placed great importance on the free energy aspect of the log P_{mic} term and are extending thermodynamic consideration of hydrophobicity.

In the present study thermodynamic aspects of partition are measured for different organic compounds, and they are used to discuss the BBB permeability.

Experimental Section

Materials. Urea, caffeine, antipyrine, thiourea, formamide, acetamide, *N*-methylnicotinamide, creatinine, 5-fluorouracil, metronidazole, and pyrimethamine were obtained from Wako chemicals and Tokyo Kasei Kogyo Co., ftorafur was from Sigma Chemical Co., and adriamycin and vincristine were from Aldrich Chemical Co., Inc. All these chemicals were used without further purification.

Methods. Micellar liquid chromatography (MLC) is best suited for quick and precise determination of micelle/water partition coefficient.[10] When the reversed-phase liquid chromatography (RPLC) was used with micellar mobile phase and octadodecylsilane (ODS) column, some solutes such as urea and formamide eluted immediately after injection because of their very small affinity to the stationary phase. This is because the solutes are too hydrophilic to be distributed to the reversed-phase ODS column. Therefore the gel filtration chromatography (GLC) was adopted with micellar mobile phase, where solutes with sizes smaller than the hole diameter are partially entrapped in the gel column, micelle molecules with larger sizes being excluded from the stationary phase.

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Two gradient liquid chromatography pumps (Waters 510) with UV detector (Waters 484) were used as apparatus. The analytical column was a gel filtration column (Shodex OHpak, Q-801s). The guard column (Shodex OHpak, Q-800p) was attached between the injector and the analytical column. These two columns were placed in a water bath that controlled the temperature within an error of ±0.1 °C. Sudan III was used as a t_0 marker. This characteristic separation system enabled to measure the retention time of hydrophilic as well as hydrophobic solutes treated here. The nonionic neutral surfactant, polyoxyethylene(23) lauryl alcohol ether (Brij35, Nacalai Tesque Inc.) was used as micelle component. The cmc of Brij35 in this solution was determined to be 0.05 mM from the conductivity measurement.[9]

A 0.01 M KH_2PO_4−0.05 M Na_2HPO_4 phosphate buffer solution (pH 7.39) was used, and 20 mM Brij35 solution was made from this phosphate buffer. These two solutions were filtered through a 0.22 μm cellulose ester membrane filter (MILLEX-GV, Millipore Corp.) and stocked for the gradient experiment. The flow rate of mobile phase was 0.5 mL/min throughout this study. The capacity factor (k') was measured for multiple injections and averaged.

Results and Discussion

The gradient chromatographic measurement was performed for four or five different concentrations, and the micelle/water partition coefficient (P_{mic}) was calculated according to eq 1

$$\frac{1}{k'} = \frac{(P_{mic} - 1)VC_m}{P_{sta}\phi} + \frac{1}{P_{sta}\phi} \quad (1)$$

where V is the partial molar volume of micelle molecule (1.18 L/mol),[11] C_m is the concentration of micelle, P_{sta} is the partition coefficient between mobile phase and stationary phase, and ϕ is the phase ratio. The linear plot of $1/k'$ against C_m was obtained experimentally with high accuracy: correlation coefficients were always higher than 0.99. The plotting of eq 1 was repeated several times after the bath temperature was changed between 20 and 60 °C. The enthalpy ($\Delta H_P°$) and entropy ($\Delta S_P°$) of partition were derived from the van't Hoff plot of these variable-temperature experiments. That is, linear plots of log P_{mic} against $1/T$ will offer $\Delta H_P°$ and $\Delta S_P°$ from the slope and the intercept as may be seen from eqs 2−4

$$\Delta G_P° = -2.303RT \log P_{mic} \quad (2)$$

$$\Delta G_P° = \Delta H_P° - T\Delta S_P° \quad (3)$$

$$\log P = -\frac{\Delta H_P°}{2.303RT} + \frac{\Delta S_P°}{2.303R} \quad (4)$$

where R is the gas constant and T is the absolute temperature. The correlation coefficients in the van't Hoff plot were above 0.95 in all cases. The hydrophobic enthalpy constant (P_H) and the hydrophobic entropy constant (P_S) were determined according to the following equations from the data obtained above by experiment.

$$\log P_{mic} = P_H + P_S \quad (5)$$

$$P_H = -\frac{\Delta H_P°}{2.303RT} \quad P_S = \frac{\Delta S_P°}{2.303R} \quad (6)$$

The properties thus determined are listed in Table 1 and a correlation matrix between them in Table 2. In Table 2, the low coefficients of log P_{mic} can be interpreted that log P_{mic} is not dependent solely on P_H or P_S, but

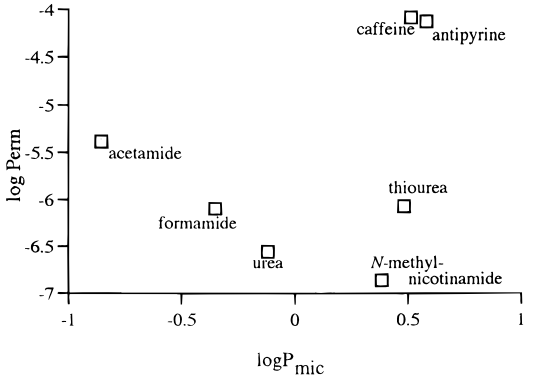

Figure 1. Correlation of log P_{mic} and the BBB permeability coefficient. The permeability data are taken from ref 3.

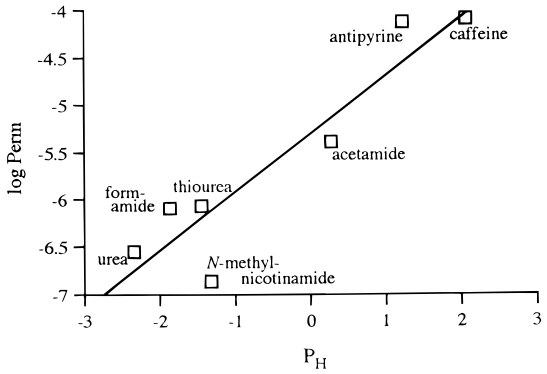

Figure 2. Correlation of P_H and the BBB permeability coefficient. The permeability data are taken from ref 3.

Table 1. Thermodynamic Parameters of Hydrophobicity[a]

compound	log P_{mic}	P_H	P_S
caffeine	0.52 ± 0.03	2.06 ± 0.02	−1.55 ± 0.02
antipyrine	0.58 ± 0.06	1.22 ± 0.04	−0.64 ± 0.04
thiourea	0.48 ± 0.13	−1.45 ± 0.09	1.93 ± 0.09
formamide	−0.35 ± 0.38	−1.87 ± 0.27	1.51 ± 0.27
acetamide	−0.86 ± 0.13	0.28 ± 0.03	−1.13 ± 0.04
N-methyl-nicotinamide	0.39 ± 0.13	−1.32 ± 0.09	1.71 ± 0.09
urea	−0.12 ± 0.25	−2.34 ± 0.18	2.22 ± 0.18
creatinine	−0.30 ± 0.95	−3.28 ± 0.67	2.99 ± 0.68
5-fluorouracil	0.21 ± 0.43	−1.24 ± 0.30	1.45 ± 0.31
metronidazole	0.54 ± 0.20	0.87 ± 0.14	−0.33 ± 0.14
pyrimethamine	2.51 ± 0.71	3.03 ± 0.50	−0.52 ± 0.50
ftorafur	0.70 ± 0.01	−0.42 ± 0.01	1.12 ± 0.01
adriamycin	1.90 ± 0.11	−7.23 ± 0.08	9.13 ± 0.08
vincristine	2.48 ± 0.16	−5.64 ± 1.12	8.13 ± 1.15

[a] The values at 37 °C are listed with standard errors.

Table 2. Correlation between Observed Parameters

	log P_{mic}	P_H	P_S
log P_{mic}	1.00	−0.20	0.49
P_H	−0.20	1.00	−0.95
P_S	0.49	−0.95	1.00

P_H and P_S contribute cooperatively to log P_{mic}. And the enthalpy-entropy compensation pattern is observed. As shown in Figure 1, the correlation between log P_{mic} and the BBB permeability coefficient (log Perm) was not good. In contrast P_H showed a good correlation in Figure 2. $1/M^{1/2}$ and log $P_{mic}/M^{1/2}$ were also tested as parameters, but they did not show any significant correlation (those correlation coefficients were 0.57 and 0.63, respectively). Analysis of another BBB permeability data[4] is indicated in Figure 3. In this case, too, it was P_H that showed a good correlation to the BBB permeability. The two results are summarized in Table 3. In both cases P_H and P_S, especially P_H, showed a good

Table 3. Relationship between the BBB Permeability Coefficient (log Perm)[3,4] and the Thermodynamic Hydrophobic Parameters

ref no.	equation[a]	n[b]	r[c]	r' [d]	F[e]	SD[f]
3	log Perm = 0.57(0.88) log P_{mic} − 5.64(0.45)	7	0.28		0.42	1.17
	log Perm = 0.62(0.10)P_H − 5.25(0.17)	7	0.93	0.92	32.95	0.45
	log Perm = −0.61(0.15)P_S − 5.24(0.24)	7	0.88	0.85	16.51	0.59
	log Perm = 0.65(0.37)P_H − 0.03(0.39)P_S − 5.29(0.20)	7	0.93	0.90	13.20	0.50
4	log Perm = 0.12(0.42) log P_{mic} − 5.92(0.61)	8	0.11		0.08	1.27
	log Perm = 0.33(0.05)P_H − 5.12(0.17)	8	0.95	0.94	52.63	0.41
	log Perm = −0.27(0.07)P_S − 4.99(0.33)	8	0.83	0.80	13.70	0.71
	log Perm = 0.57(0.12)P_H + 0.22(0.11)P_S − 5.33(0.17)	8	0.97	0.95	42.05	0.33

[a] In parentheses are listed standard errors. [b] Number of data. [c] Multiple correlation coefficient. [d] Multiple correlation coefficient adjusted for the degree of freedom. [e] Variance ratio. [f] Standard deviation.

relationship to the BBB permeability although log P_{mic} failed. This fact indicates that the permeation process, which is related to the partition from the aqueous phase to the BBB lipophilic phase and may be governed by the *in vivo* partition coefficient, is successfully simulated by endo- or exothermic properties accompanying partition in the micelle/water model system. This explanation is very interesting because hydrogen-bonding potential is reported to be an important parameter for the peptide permeability across the BBB.[6] That is, the P_H dependency may be a result of formation or breaking of a hydrogen bond on transfer from the aqueous phase to the BBB lipophilic phase. In this sense, it is suggested that the lipophilicity *in vivo* which governs the permeability is dominated by hydrogen-bonding potential, whereas in the model micelle/water system the partition is dominated by hydrogen-bonding potential related to the P_H term as well as by some other factors which relate to the P_S term. So far thermodynamics of solute partitioning and receptor binding has been studied,[12,13] in which the enthalpy or entropy contribution has been discussed. However it is not clear at present what is dominant in the P_S term, but molecular mobility (degree of freedom) may be pointed out as a candidate factor. The success of heptane/ethylene glycol partition coefficient and failure of octanol/water partition coefficient to correlate to the BBB permeability may also be explained by difference in the relative contribution of the enthalpy and entropy parts in the log P terms. In the QSAR analysis of Table 3, the entropy/enthalpy ratio α,[7] which is defined as $\alpha = b/a$ in the regression analysis using P_H and P_S as dual independent parameters (eq 7), is smaller than 1.0.

$$\log \text{Perm} = aP_H + bP_S + C \qquad (7)$$

This ratio depicts the relative contribution of the entropy term in the biological response as seen from the micelle/water system adopted for a reference.[7] This factor is expected to change depending on the property of interface to be permeated. To examine such dependency, other permeability data need to be analyzed.

Among the compounds treated in the present work, adriamycin and vincristine are considered to be transported by P-glycoprotein.[14] But the BBB permeability coefficients of these two compounds[4] appear on the same linear relationship as observed with others in Figure 3 (□). The BBB permeability data was measured by using a single-time-point method.[4] In the mass balance equation in this method the efflux of compounds from brain to blood is taken to be negligible. The supposed function of P-glycoprotein is an efflux pump of drug from brain to blood. Therefore the permeability data reported is considered not to reflect the P-glycoprotein effect. Another study[15,16] supports this interpretation. The

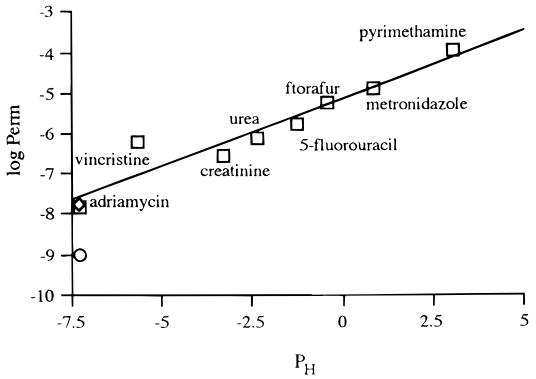

Figure 3. Correlation of P_H and the BBB permeability coefficient. The symbol ○ indicates the effect of P-glycoprotein (P-gp) on the permeability of adriamycin. (□) Data from ref 4. (○) Data from ref 15, at ATP normal level where P-gp functions as a drug efflux pump. (◇) Data from ref 15, under ATP depletion where P-gp does not function.

P-glycoprotein function has been confirmed to be ATP-dependent, so when ATP is run out, the efflux system of P-glycoprotein does not work. As shown in Figure 3, the permeability coefficient data of adriamycin obtained under the depletion of ATP (◇) was very close to the other permeability data (□). On the contrary, when ATP was at a normal level (○), that is when P-glycoprotein can take effect, the permeability coefficient of adriamycin was low and did not follow the linear relationship to the P_H. This fact means that the adriamycin permeates into brain by the process of passive transportation following P_H dependency, but owing to the P-glycoprotein function as an efflux pump, the uptake of adriamycin to the brain will be suppressed. In this manner the hydrophobic constant P_H is valuable for the discussion of presence or absence of specific mechanism such as transportation by P-glycoprotein.

As discussed above, a simple relationship was found between the BBB permeability[3,4] and the hydrophobicity constant P_H. This relationship is valuable to clarify the transport mechanism *in vivo*. The decisive role of the enthalpy term P_H suggests that an endo/exo thermal effect is a driving force of the BBB transport phenomena. In this sense the hydrogen-bonding potential may also affect the permeability through this endo/exo thermal effect observed in the model partitioning system. In this way thermodynamic aspects of partition are supported to be important for the discussion of the BBB permeability of drugs.

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