Notes

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Binding Parameters of Theophylline and Aminophylline to Bovine Serum Albumin

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The interaction of theophylline and bovine serum albumin (BSA) was investigated by using the equilibrium dialysis method. The experiments were carried out at 5°C and at pH 6.85 using 1/15 M phosphate buffer. The binding parameters determined are as follows; $n_1 = 0.48$, $n_2 = 1.34$, $K_1 = 2.89 \times 10^3\ M^{-1}$, and $K_2 = 4.03 \times 10^2\ M^{-1}$. The interaction of aminophylline with BSA was also investigated in the same manner. The binding parameters are as follows; $n_1 = 0.43$, $n_2 = 1.29$, $K_1 = 2.74 \times 10^3\ M^{-1}$, and $K_2 = 5.61 \times 10^2\ M^{-1}$. These parameters are calculated on the basis of theophylline content in aminophylline, which is a 2 : 1 complex of theophylline with ethylenediamine. There was little difference in binding parameters between theophylline and aminophylline. This is to be expected in view of the findings that under the experimental conditions used, theophylline does not interact significantly with ethylenediamine, and the latter shows essentially no interaction with BSA.

Keywords——theophylline; aminophylline; bovine serum albumin; binding parameter; Scatchard plot; equilibrium dialysis

It is well known that the binding of drugs with proteins has a great influence not only upon the distribution of the drugs in the body but also upon their patterns of metabolism and excretion. Further, from the standpoint of drug efficacy, only that portion of a drug which is not bound with plasma proteins is generally bio-active. Thus, the study of the binding characteristics of medical drugs to proteins is an important field in drug research.

There are some quantitative reports[1-4] on the binding of xanthine compounds, such as 8-chlorotheophylline and 8-nitrotheophylline, to proteins, whereas the binding of theophylline, which is widely used as a drug, to proteins was studied only semi-quantitatively in 1962 by Eichman *et al.*[1] This was because the interaction is weak and the exact binding parameters are not available easily. We have now carried out a quantitative study on the interaction of theophylline with bovine serum albumin (BSA), using the equilibrium dialysis technique. The interaction of aminophylline with BSA was also investigated.

Experimental

Materials——Bovine serum albumin, fraction V (BSA) (purchased from Armour Pharmaceutical Co., U.S.A.), was used without further purification; its molecular weight was assumed to be 66000. Reagent grade KH_2PO_4 and Na_2HPO_4 were used to prepare pH 6.85 buffer solution (1/15 M). Theophylline was used after purification by recrystallization from water. Aminophylline JP was used without further purification. Ethylenediamine was used after purification by distillation. Other chemicals were of reagent grade. Water that had been deionized and doubly distilled in all-glass apparatus was used. All drug and BSA solutions were prepared immediately before use for equilibrium dialysis, using the pH 6.85 buffer solution mentioned above.

Equilibrium Dialysis——i) Theophylline–BSA System: Dialysis cells of acrylic resin, similar to those used by

Goto *et al.*,[5] were used for the equilibrium dialysis experiment. Visking cellulose membrane was used for dialysis and before use it was boiled in distilled water for more than 12 h. The cellulose membrane thus treated was inserted between the dialysis cells with two silicon packing and fixed with metallic screws. Then 4 ml aliquots of BSA solution (2.90×10^{-4} M) and drug solution ($0.6—300 \times 10^{-5}$ M) were added to opposite compartments of the apparatus. The cells were shaken at a constant temperature for 18 h in a water bath (Tokyo Rikakikai Co., Thermister Tempett T-80). After equilibrium had been reached, the drug concentrations were determined by measurement of the absorbance at 272 nm in a Shimadzu spectrophotometer (model 200) using 1/15 M phosphate buffer as a blank.

ii) Ethylenediamine–BSA System: Equilibrium dialysis was carried out at 5 °C with 4 ml each of 7.5×10^{-4} M ethylenediamine and 2.90×10^{-4} M BSA. After equilibrium, 3 ml aliquots were removed; each aliquot was mixed with 0.5 ml of 2.8×10^{-3} M $CuCl_2$ (ethylenediamine: $Cu^{2+} = 2:1$, assuming that ethylenediamine does not bind to BSA), then the pH was adjusted to 10.5 with 0.1 N NaOH and the volume to 5 ml. The absorption spectrum of the solution was recorded from 500 to 700 nm (the chelate compound with a molar ratio of 2:1 has λ_{max} of 550 nm). Ethylenediamine was determined by comparing the spectrum with standard absorption spectra which were recorded in advance with various known concentrations of ethylenediamine.

Results and Discussion

Figure 1 shows a Scatchard plot of the results for the binding of theophylline to BSA obtained by the equilibrium dialysis of theophylline at 5 °C, where r is the ratio of the bound drug to protein (mol/mol), and C_f is the free drug concentration. The plots are apparently curved, suggesting the presence of at least two types of independent binding sites. On the assumption that each BSA molecule has two types of binding sites, the data were analysed according to a Langmuir-type adsorption isotherm (Eq. 1);

$$r = \frac{n_1 K_1 C_f}{1 + K_1 C_f} + \frac{n_2 K_2 C_f}{1 + K_2 C_f} \tag{1}$$

where K_1 and K_2 are the association constants of the primary (1) and secondary (2) binding sites, respectively, and n_1 and n_2 are the numbers of primary and secondary sites, respectively.

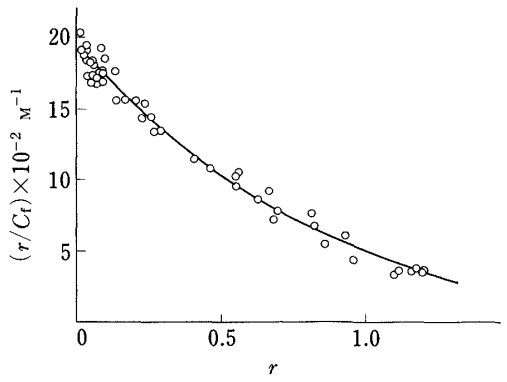

Fig. 1. Scatchard Plots for the Binding of Theophylline to BSA

All points are experimental data while the solid line was computed from the binding parameters.

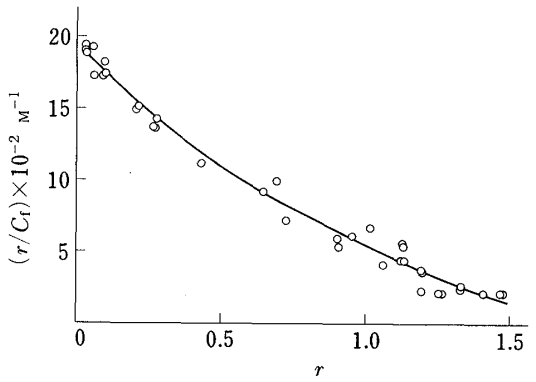

Fig. 2. Scatchard Plots for the Binding of Aminophylline to BSA

The plot is based on theophylline content in aminophylline. All points are experimental data while the solid line was computed from the binding parameters.

TABLE I. Binding Parameters for the Interaction of Theophylline and Aminophylline[a] with BSA at 5 °C and pH 6.85

	n_1	n_2	$K_1 \times 10^{-3}$ M^{-1}	$K_2 \times 10^{-2}$ M^{-1}
Theophylline	0.48	1.34	2.89	4.03
Aminophylline	0.43	1.29	2.74	5.61

a) Binding parameters calculated on the basis of theophylline content in aminophylline.

The most probable values for n_1, n_2, K_1, and K_2 were calculated by a repetitive least-squares method, (SALS program) on an NEC ACOS system 1000 at the Computer Center of Osaka University and are shown in Table I. The solid line is the simulated line obtained by using the parameters given in Table I. For comparison, the binding parameters at 9 °C in pH 6.85 phosphate buffer estimated by Eichman *et al.*[1] by the equilibrium dialysis method were as follows: $n_1=1$, $n_2=2$ (these values were assumed), $K_1=1.61\times10^3\,\text{M}^{-1}$, and $K_2=9\times10\,\text{M}^{-1}$.

As theophylline is often used in the form of aminophylline, a complex of theophylline with ethylenediamine in a molar ratio of 2 : 1,[6] the interaction of aminophylline with BSA was studied by the equilibrium dialysis method under conditions similar to those used in the study of theophylline. The experimental results are shown in Fig. 2 as a Scatchard plot. In practice, the binding amount of theophylline to BSA was examined, and the Scatchard plot was prepared on this basis. The data for aminophylline closely resemble those for theophylline. On the assumption of the presence of two types of independent binding sites, the binding parameters were calculated in the same way as for theophylline. The binding parameters of theophylline and aminophylline (Table I) are very similar. From Table I, it appears that the values of n_1 are smaller than 1. This might be because BSA is not molecularly homogeneous with respect to binding properties.

Next, the interaction of ethylenediamine and BSA was studied using the method described in Experimental. When ethylenediamine in the drug compartment was determined after equilibrium had been reached, the concentration was unchanged after correction for the dilution. It is, therefore, clear that there is essentially no interaction between the two substances. Further, it has been reported that there is practically no interaction between theophylline and ethylenediamine around pH 6.8.[6,7] These facts, together with the results in Fig. 2, indicate that there is practically no difference in the binding of theophylline to BSA whether theophylline is used in the free form or as aminophylline.

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