

## Binding Characteristics of Tryptophan Metabolites to Bovine Serum Albumin

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The interaction between tryptamine, 3-indoleacetic acid and 5-hydroxyindole-3-acetic acid and bovine serum albumin (BSA) was investigated using the equilibrium dialysis technique. The apparent binding constant was determined assuming the equivalence and independence of the binding sites on the BSA molecule. The binding constants of tryptamine, 3-indoleacetic acid and 5-hydroxyindole-3-acetic acid with a single binding site are  $1.07 (0.14) \times 10^4 \text{ M}^{-1}$ ,  $1.73 (0.15) \times 10^4 \text{ M}^{-1}$  and  $2.02 (0.15) \times 10^4 \text{ M}^{-1}$  at 25 °C at pH 6.5, respectively. The affinity of the tryptophan metabolites to BSA strengthened with progression of the metabolic process, and it reached a maximum at a relatively narrow pH region of 6 to 8.

**Keywords** tryptamine; 3-indoleacetic acid; 5-hydroxyindole-3-acetic acid; bovine serum albumin; binding constant; equilibrium dialysis; tryptophan metabolite

### Introduction

Tryptophan metabolites play important physiological functions and their crystal structures have been determined extensively.<sup>1)</sup> However, very few investigations have been reported regarding the interaction between tryptophan metabolites and serum albumin. It is important to clarify the binding characteristics of tryptophan metabolites to bovine serum albumin (BSA), because BSA binds many drugs and/or low molecular weight bioactive substances.<sup>2)</sup> This paper describes studies of this interaction using the equilibrium dialysis technique, and presents some fundamental information concerning the binding characteristics of tryptophan metabolites, tryptamine, 3-indoleacetic acid and 5-hydroxyindole-3-acetic acid to BSA.

### Materials and Methods

BSA (lot No. 86) was obtained from Seikagaku Kogyo Co., Ltd., Tokyo. Tryptamine (lot No. ECN3352), 3-indoleacetic acid (lot No. SAN0454) and 5-hydroxyindole-3-acetic acid (lot No. AY01) and other reagents of the highest quality were obtained from Wako Pure Chemical Ind., Ltd., Osaka. The equilibrium dialysis cell EC-O and membranes were obtained from Sanplatec Co., Ltd., Osaka. The binding reaction was performed as follows: 500  $\mu\text{l}$  of the reaction mixture in 0.1 M sodium phosphate buffer, at appropriate pH, 0.3 mM ethylenediaminetetraacetic acid (EDTA), containing  $2.5 \times 10^{-5} \text{ M}$  BSA and various concentrations of ligands ( $0.125$ – $1.13 \times 10^{-4} \text{ M}$ ) were equilibrated against 500  $\mu\text{l}$  of the above buffer. The equilibrium cell was shaken for 20 h at 4 °C and 25 °C regulated within 0.1 °C in the dark. Tryptamine and other metabolites used in this study have similar fluorescence characteristics at the wavelength of 335 nm (3-indoleacetic acid, 5-hydroxyindole-3-acetic acid) and 345 nm (tryptamine), when they were excited at 380 nm. The concentrations of free ligands were determined fluorometrically using a Hitachi 850 spectrofluorometer from the calibration curves of the known concentrations. The adsorption of the ligands to the dialysis membrane was also determined and corrected. The concentration of BSA was determined spectrophotometrically using  $E_{1\%}^{1\text{cm}} = 6.54$  at 280 nm and a molecular weight of 66300.<sup>3,4)</sup> The binding constants and the number of binding sites were determined by the double-reciprocal method described by Hughes and Klotz<sup>5)</sup> using the following expression:

$$\frac{1}{r} = \frac{1}{nK} \left( \frac{1}{C} \right) + \frac{1}{n} \quad (1)$$

where  $r$  is the number of moles of ligands bound per mol of BSA,  $n$  is the number of binding sites,  $K$  is the binding constant and  $C$  is the concentration of free ligands. The solvent and the sample solutions were degassed carefully under vacuum before the reaction mixture was prepared.

### Results and Discussion

The apparent binding constants at pH 6.5 at 25 °C are summarized in Table I. The binding constants of trypto-

phan metabolites are a little lower than the value for binding of tryptophan to human serum albumin (HSA) with  $K = 6.3 \times 10^4 \text{ M}^{-1}$ .<sup>6)</sup> The affinity of tryptamine is weaker than the indolealkylacetic acids, 3-indoleacetic acid and 5-hydroxyindole-3-acetic acid, both of which are progressive metabolic products of the indolealkylamines. The affinity of 5-hydroxyindole-3-acetic acid is the strongest of all. The number of the binding site of one indicates the specific binding of these metabolites to BSA. By comparing the substituent groups on the indole ring, the carboxyl groups of 3-indoleacetic acid and 5-hydroxyindole-3-acetic acid may have an important role in the affinity of these metabolites to BSA. The negatively charged carboxyl group of the metabolite might be located in the vicinity of the positively charged arginyl and/or lysyl side chains of BSA, which project more from the surface of BSA than the negatively charged glutamyl and aspartyl side chains.<sup>7)</sup> The thermodynamic parameters are listed in Table II. Binding free energy changes are in the range of  $-5$  to  $-6 \text{ kcal mol}^{-1}$ . The binding of 3-indoleacetic acid and 5-hydroxy-3-indoleacetic acid are characterized by an increase in entropy and enthalpy. On the other hand tryptamine binding is

TABLE I. Binding Constants of Binding of the Tryptophan Metabolites to BSA at pH 6.5 at 25 °C

	$K(\text{S.D.}) \times 10^{-4} (\text{M}^{-1})^a$
Tryptamine	1.07 (0.14)
3-Indoleacetic acid	1.73 (0.15)
5-Hydroxyindole-3-acetic acid	2.02 (0.20)

<sup>a)</sup> Binding constants were determined from Eq. 1 assuming the number of binding sites,  $n = 1.0$ , since the intercept ( $1/n$ ) of 1.0 was the optimum for fitting the equation. The standard deviations (S.D.) were determined from repetitions of the equilibrium dialysis experiments.

TABLE II. Thermodynamic Parameters<sup>a)</sup> of Binding of Tryptophan Metabolites to BSA at pH 6.5 at 25 °C

	$\Delta G$ ( $\text{kcal mol}^{-1}$ )	$\Delta H$ ( $\text{kcal mol}^{-1}$ )	$\Delta S$ (e.u.)
Tryptamine	-5.49	-2.01	11.7
3-Indoleacetic acid	-5.78	2.70	28.5
5-Hydroxyindole-3-acetic acid	-5.87	1.03	23.2

<sup>a)</sup> These parameters were determined from the average values of the binding constants at 25 °C and 4 °C.

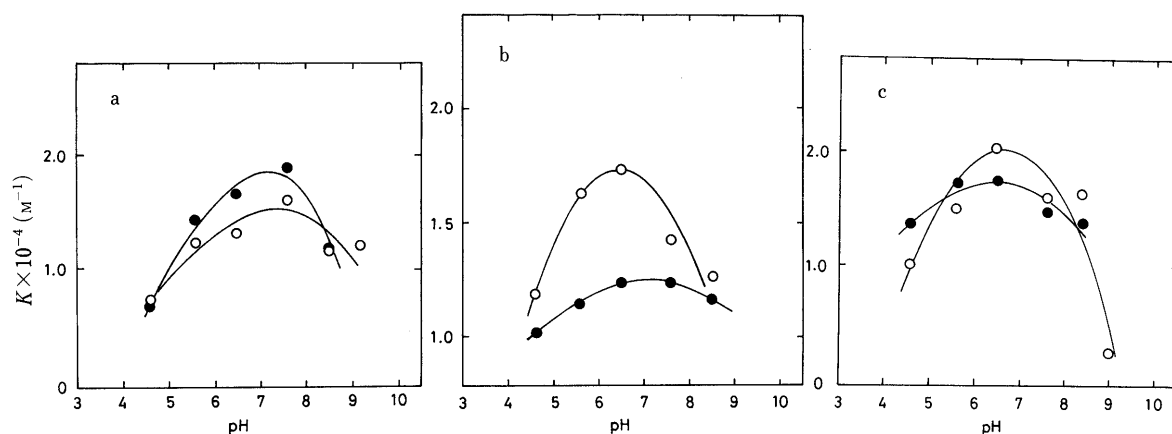


Fig. 1. Values of the Binding Constants as a Function of pH for a, Tryptamine; b, 3-Indoleacetic Acid; c, 5-Hydroxyindole-3-acetic Acid, as Determined by the Equilibrium Dialysis

Temperatures were  $\circ$ , 25°C and  $\bullet$ , 4°C. The standard deviations of the values were within 15%.

characterized by an increase in entropy and a decrease of enthalpy. The positive entropy and enthalpy changes of 3-indoleacetic acid and 5-hydroxyindole-3-acetic acid suggest that these metabolites could bind to the hydrophobic pocket on the BSA molecule.<sup>8)</sup> In the case of tryptamine, its binding to the hydrophobic pocket could be also suggested by the increase in entropy, and the negative enthalpy change might be explained the hydrogen bond formation of the amino group of tryptamine in the hydrophobic binding pocket.<sup>8)</sup> Figures 1a—c show the pH dependence of the apparent binding constants of tryptamine, 3-indoleacetic acid and 5-hydroxyindole-3-acetic acid at 25°C and 4°C. The binding of 3-indoleacetic acid and 5-hydroxyindole-3-acetic acid reached a maximum at the pH range of 6 to 7 and decreased both in acid the alkaline pH's. This feature is conspicuous at 25°C, and the affinity at 4°C is generally weaker than at 25°C. On the other hand, as shown in Fig. 1a, the binding of tryptamine reached a maximum at a low alkaline pH of 7—8, and also decreased in acid and alkaline conditions. The affinity of tryptamine is stronger at low temperatures. The decrease of the affinity of these metabolites to BSA at both acidic and alkaline

pH's could be explained mainly by the acid and alkaline conformational change in the BSA molecule. This is because the overall pH profiles of the binding of the metabolites resemble each other in spite of their different charges, a further partial opening of the albumin molecule occurs both at an acidic pH of 4—4.5 causing exposure of the carboxy groups, and at more than pH 8 causing alkaline expansion.<sup>2)</sup> The binding of the tryptophan metabolites to BSA might be affected by subtle isomerizations of BSA molecule.

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