Effect of Inorganic Anions on the Binding of Thyroxine by Bovine Serum Albumin

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The fluorescence method was used to investigate the effect of inorganic anions, SCN^-, I^-, ClO_4^- and Br^- on the binding of thyroxine (T_4) by bovine serum albumin (BSA). The apparent binding constants were evaluated in 0.1 M sodium phosphate buffer, pH 7.4 at 25 °C containing various concentrations of the anions up to 0.15 M. Their values decreased at high anion concentrations. The effectiveness of anions in reducing T_4 binding was in the following order: $SCN^- > I^- > ClO_4^- > Br^-$. Thermodynamic parameters determined in the presence of 0.1 M SCN^- suggested that the hydrophobic interaction between T_4 and BSA could be reduced by these inorganic anions.

Keywords thyroxine; bovine serum albumin; inorganic anion; thyroid hormone; hormone binding

Introduction

The interaction of thyroxine (T_4) with serum albumin has been studied for its biological importance.[1-11] As the results, it has been indicated that there is a single strong binding site involving the positively charged active lysine residue (Lys-412 in bovine serum albumin (BSA) and Lys-414 in human serum albumin (HSA)[12]). Tabachnick[8,13] reported that the anionic organic compounds fatty acids, dodecyl sulfate or 2,4-dinitrophenol might be capable of displacing T_4 from its binding sites on albumin, and that T_4 binding by HSA was reduced by 50% in the presence of 0.1 M NaCl at 30 °C and pH 7.4. This strongly suggests an influence of these anions on the electrostatic interaction between T_4 and albumin. To obtain further information about the effect of small anions on the binding of T_4 by albumin, the binding properties of T_4 to BSA were investigated here in the presence of various inorganic anions.

Materials and Methods

BSA (lot No. 86) was obtained from Seikagaku Kogyo Co., Ltd. T_4 (lot No. LKP0093), sodium bromide (lot No. LKN2283), sodium iodide (lot No. DSR2981), sodium perchlorate (lot No. DSM0624) and sodium thiocyanate (lot No. SAH2184), and other reagents of the highest quality were obtained from Wako Pure Chemical Industries, Osaka. Fluorescence measurements were performed with a Hitachi 850 spectrofluorometer. Temperature of the sample was controlled by a hollow cell holder through which water from a constant temperature bath regulated within 0.1 °C was circulated and was measured directly by a Takara thermister D641. The apparent binding constants were evaluated from the fluorescence titration curves according to the method of Attallah and Lata,[14]

$$K=\frac{Q_f}{(1-Q_f)(T_t-nQ_fP_t)} \quad (1)$$

$$Q_f=\frac{f_o-f_m}{f_o-f_r} \quad (2)$$

where K is the apparent binding constant, Q_f is the ratio of quenching at a point on the quenching curve to the maximum quenching, T_t is the total concentration of T_4 added, P_t is the total BSA concentration, n is the number of T_4 bound to a BSA molecule, f_o is the fluorescence intensity of the albumin solution without added T_4, f_m is the measured intensity of the sample added with T_4, and f_r is the rest of the fluorescence intensity not quenchable with T_4. In data analysis, the best fit to the fluorescence data was obtained by setting $n=1$. In a typical experiment, aliquots (0.5—1.0 μl) of 0.2 mM T_4 were added to 200 μl of 2 μM BSA solution in 0.1 M sodium phosphate buffer, pH 7.4 containing various amounts of anions in a $0.5 \times 0.5\,cm^2$ quartz micro cuvette under stirring. The observed relative intensity was corrected for dilution of the albumin. The BSA concentrations was determined spectrophotometrically using $E_{1cm}^{1\%}=6.54$[9] at 280 nm and the molecular weight of 66300[15] in sodium phosphate buffer pH 7.4. Ultraviolet absorption measurements were made in a Hitachi 557 double-beam spectrophotometer.

Results and Discussion

Figure 1 shows the effect of various inorganic anions, SCN^-, ClO_4^-, Br^- and I^-, on the fluorescence intensity of BSA. Under these experimental conditions, the protein fluorescence was quenched by SCN^-, Br^- and I^-, but not by ClO_4^-. In the presence of 0.20 M anions, the fluorescence was quenched in the following order: I^- (40%) > Br^- (10%) > SCN^- (5%) > ClO_4^-. The fluorescence spectra at extremely high SCN^- ion concentrations are shown in Fig. 2. In the presence of 4.0 M SCN^-, the maximum fluorescence intensity decreased about 60% as compared with that in the absence of anions, and the wavelength at the fluorescence peak shifted about 15 nm to the shorter wavelength. This remarkable quenching suggests that the conformational change of BSA could occur at SCN^- concentrations of more than 1 M, because inorganic salts like LiBr, KSCN, KI or $CaCl_2$ cause conformational changes of proteins at high salt concentrations.[16-18] This result, therefore,

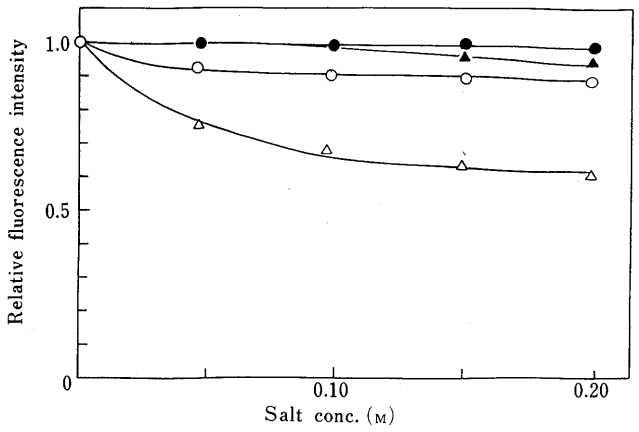

Fig. 1. Effect of Salt Concentrations on the Fluorescence Intensity of BSA in 0.1 M Sodium Phosphate Buffer, pH 7.4 at 25 °C

Fluorescence excitation and emission wavelengths were 280 and 343 nm, respectively. BSA concentration was 2.00 μM. ○, NaBr; ▲, NaSCN; △, NaI; ●, $NaClO_4$.

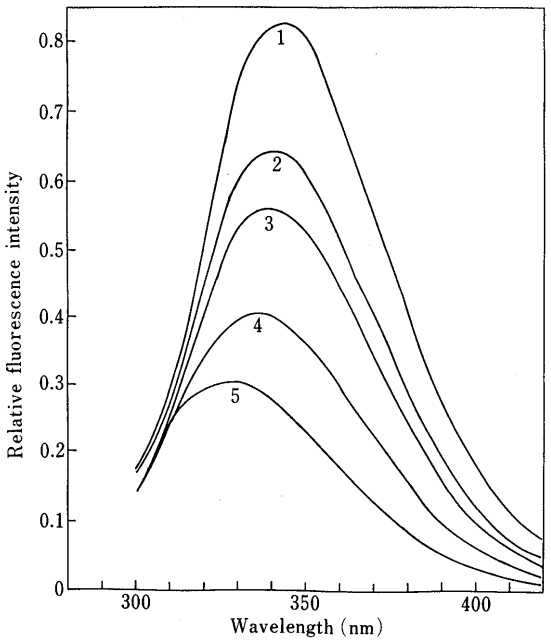

Fig. 2. Fluorescence Spectra of BSA in the Presence of NaSCN in 0.1 M Sodium Phosphate Buffer, pH 7.4 at 25 °C

BSA concentration was 2.00 μM. Fluorescence excitation wavelength was 280 nm. 1, salt-free; 2, 1.0 M; 3, 2.0 M; 4, 3.0 M; 5, 4.0 M.

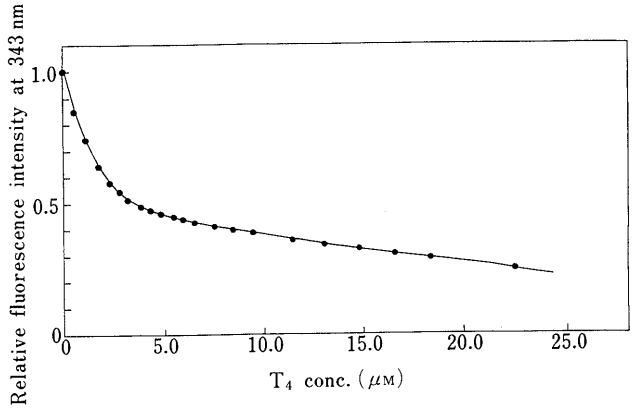

Fig. 3. Relative Fluorescence Intensity of BSA in the Presence of 0.10 M NaSCN against T_4 Concentrations in 0.1 M Sodium Phosphate Buffer, pH 7.4 at 25 °C

Fluorescence excitation and emission wavelengths were 280 and 343 nm, respectively. BSA concentration was 2.00 μM.

indicates that little conformational change of BSA occurs at concentrations of SCN^- lower than 0.20 M, although the existence of a minor effect of SCA^- on the conformation of BSA can not be completely neglected. It may be reasonable to consider that ClO_4^-, I^- and Br^- cause little conformational change of BSA at concentrations of these anions below 0.2 M, because the ability of denaturants of these anions for proteins is lower than that of SCN^-. A large fluorescence quenching observed in the presence of 0.2 M I^- (Fig. 1) might be explained as ordinal quenching effect by halogen anions.[19] The typical fluorescence titration curve of BSA in the presence of 0.1 M SCN^- ions against T_4 concentrations is shown in Fig. 3. The curve reflects the binding of T_4 molecules to a BSA molecule, and the apparent binding constant was determined from the curve. In the presence of 0.1 M SCN^-, the average binding

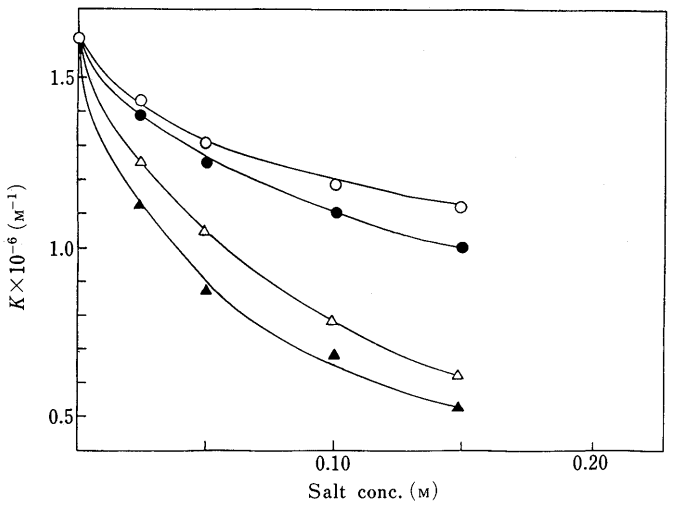

Fig. 4. The Binding Constants for T_4 Binding to BSA in the Presence of Various Concentrations of Salts

Conditions were the same as in Fig. 3. ○, NaBr; ●, $NaClO_4$; △, NaI; ▲, NaSCN.

constant was obtained from five repetitions of the fluorescence titrations as $K=0.69\ (0.11)\times 10^6\ M^{-1}$. Similar titration curves were also obtained in the presence of other anions (data not shown). The anion concentration dependence of apparent binding constants is shown in Fig. 4. The apparent binding constant was reduced in the presence of anions in the following order: $SCN^- > I^- > ClO_4^- > Br^-$; this corresponds to the reverse order of Hofmeister's series which is proportional to the ability of hydration and means that inhibition of T_4 binding by BSA was caused more effectively by less hydrated anions. This order may also be related to the binding ability of these anions to albumin, because the binding constants for SCN^-, I^- and Cl^- binding to HSA are 3.35×10^4, 6.15×10^3 and $7.2\times 10^2\ M^{-1}$ respectively.[20] The binding of T_4 by BSA involves, in part, the electrostatic interaction between negatively charged T_4 and a positively charged Lys-412 in the binding site.[12] The radius of Br^- ion, the smallest anion used in this study, is 1.95 Å,[21] therefore, the radius of the hydrated Br^- ion is about 3 Å. The radius of the cavity for the specific binding site for T_4 may be near or over 4.5 Å which is half the interatomic distance between iodine atoms at positions 3′ and 5′ in the T_4 molecule.[4] Based on this information, the reduction of the binding of T_4 to BSA in the presence of inorganic anions can be explained by weakening of the electrostatic interaction between T_4 and BSA and by the sterical hindrance at the T_4 binding site caused by inorganic anions. The van't Hoff plot for T_4 binding to BSA in the presence of 0.1 M SCN^- ion is shown in Fig. 5. $\ln K$ is linearly related to $1/T$ in this temperature range. The enthalpy change, ΔH, was evaluated from the slope of the linear line. The thermodynamic parameters in the presence and absence of SCN^- ions are summarized in Table I. The values in the absence of anions are similar to those reported for T_4 binding to HSA at pH 7.4 at 30 °C: $\Delta G=-8.40$ kcal mol^{-1}, $\Delta H=-2.02$ kcal mol^{-1} and $\Delta S=21$ e.u.[8] In both HSA and BSA, the principal contribution to the free energy of binding of T_4 to albumin is derived from the large increase of ΔS. Previous results[5,7,11] have shown that hydrophobic interaction involving iodinated two benzene rings and electrostatic interactions involving

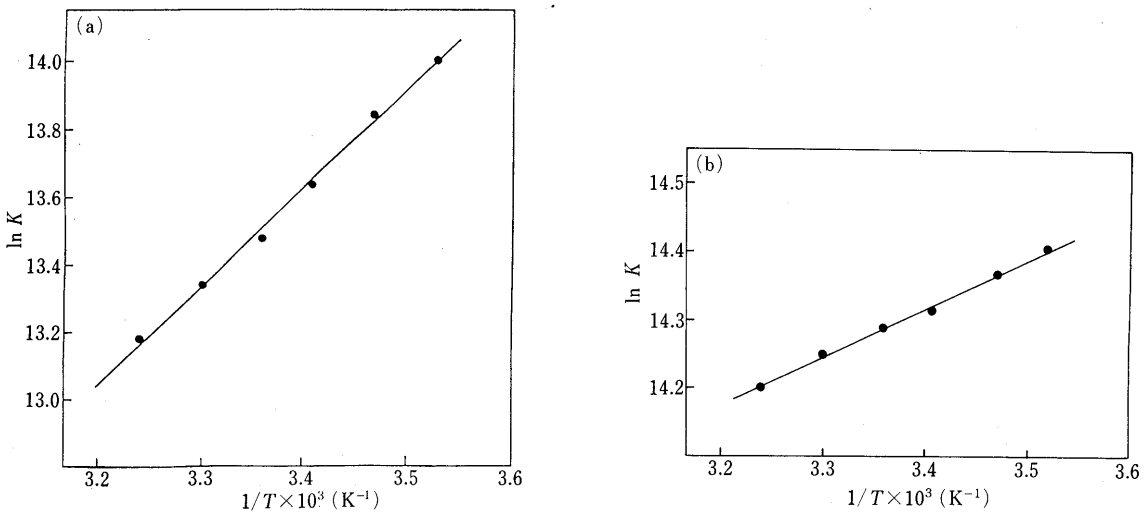

Fig. 5. Van't Hoff Plot of the Binding Constants for T_4 Binding to BSA in 0.1 M Sodium Phosphate Buffer, pH 7.4
a, in the presence of 0.1 M NaSCN; b, NaSCN-free.

TABLE I. Thermodynamic Parameters for T_4 Binding to BSA in 0.1 M Sodium Phosphate Buffer, pH 7.4 at 25 °C, in the Presence and Absence of NaSCN

	ΔG (kcal/mol)	ΔH (kcal/mol)	ΔS (e.u.)
In the presence of 0.1 M NaSCN	−7.96 (0.09)	−5.11	9.6
NaSCN-free	−8.48 (0.06)	−1.37	23.9

Standard deviations (S.D.) were obtained from five fluorescence titrations.

charged groups of T_4 are required for the strong interaction between T_4 and HSA. As suggested by Kauzmann,[22] ΔS and ΔH may be positive and negative, respectively, in the electrostatic interaction, and both may be positive in the hydrophobic interaction. Therefore, the large positive values of ΔS in the absence of SCN^- ions might be explained by hydrophobic and electrostatic interactions between T_4 and BSA as in the case of HSA.[8] On the other hand, in the presence of SCN^- ions, both ΔH and ΔS decreased as compared with those in the absence of the anions. These results may, in part, be explained by the decrease of hydrophobic interactions which cause the decrease of both ΔH and ΔS, since the binding of the hydrated SCN^- ions to BSA may lower the hydrophobicity of the binding site. The change in the thermodynamic parameters suggests that the reduction of the hydrophobic interaction should also be taken into consideration as one reason for the apparent decrease of binding constants by inorganic anions, I^-, ClO_4^- and Br^- ions as well as SCN^- ions.

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