Thyroxine Binding Properties of Glycosylated Bovine Serum Albumin

Nobuo OKABE* and Misa HOKAZE

Faculty of Pharmaceutical Sciences, Kinki University, Kowakae 3–4–1, Higashiosaka, Osaka 577, Japan. *Received May 13, 1992*

Thyroid hormone, thyroxine (T_4) binding properties of glycosylated bovine serum albumin (G-BSA), and intact BSA were studied by the fluorescence method. The apparent binding constants for intact BSA were 0.8 (0.16) $\times 10^6\ M^{-1}$ at pH 5.0 and 2.18 (0.06) $\times 10^6\ M^{-1}$ at pH 9.5 at 25 °C. T_4 binding for G-BSA was independent of pH and the apparent binding constant was $1.4 \times 10^6\ M^{-1}$. Thermodynamic parameters were also evaluated from the Van't Hoff plots of the apparent binding constants at pH 7.4 and 8.5. At both pH's, the free energy, enthalpy and entropy changes were almost the same for both G-BSA and BSA.

Keywords thyroid hormone; thyroxine; hormone binding; bovine serum albumin; glycosylated bovine serum albumin

Introduction

About 8% of the total albumin molecules in the normal individual, and more in the diabetic, are glycosylated.[1] It is known that the affinity of some drugs such as bilirubin and *cis*-parinaric acid for glycosylated human serum albumin (G-HSA) is reduced relative to intact human serum albumin (HSA), and it is also known that the principal site of the glycosylation of HSA is Lys525.[2] However, the binding properties of the glycosylated albumin for many drugs or chemicals are still unclear. In this study, we aimed to investigate the thyroid hormone, thyroxine (T_4), binding properties of G-BSA by the fluorescence method. The result will provide some fundamental information about T_4 circulation in the blood, especially its relation with glycosylated albumin.

Materials and Methods

Bovine serum albumin (BSA) (lot No. 86) was obtained from Seikagaku Kogyo Co., Tokyo, and glycosylated BSA (lot No. 29F3933, 1.25 mol hexose per mole BSA) was from Sigma Chemical Co., St. Louis. Other reagents were of the highest quality available and obtained from Wako Pure Chem. Ind., Osaka.

The apparent binding constants and the number of binding sites for the complex formation between the albumin and T_4 were evaluated from the quenching data of the protein fluorescence with increasing T_4 concentration.[3] The data were analyzed according to Attalla and Lata[4] assuming the equivalence and independence of the binding sites,

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

$$Q_f=\frac{f_o-f_m}{f_o-f_r}$$

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 added T_4, P_t is the total albumin concentration, n is the number of T_4 bound to an albumin molecule, f_o is the total fluorescence intensity of an albumin solution without T_4, f_m is the measured fluorescence intensity of the sample in the presence of T_4, and f_r is the residual fluorescence intensity non-quenchable by T_4. In this study, the best fit to the fluorescence data was obtained by the setting $n=1$.

Fluorescence measurements were performed with a Hitachi 850 spectrofluorometer. The temperature of the sample was controlled by the use of a hollow cell holder through which water from a constant temperature bath regulated within 0.1 °C was circulated. Temperature in the cell was measured directly by a Takara thermister D641. The fluorescence excitation and emission wavelengths were 280 and 340 nm, respectively. In a typical experiment, aliquots (0.5—1.0 μl) of 0.2 mM T_4 were added to 200 μl of 2 μM albumin solution in 0.1 M sodium phosphate buffer at appropriate pH's. The observed fluorescence intensity was corrected to the concentration of the albumin. BSA and G-BSA concentrations were determined spectrophotometrically using $E_{1\%}^{1cm}=6.54$ at 280 nm[5] and the molecular weight of 66300,[6] which were confirmed by the Lowry method.[7]

Results and Discussion

Figure 1 shows the effect of pH on the T_4 binding to BSA and G-BSA. The binding constant increased with increasing pH, from 0.83 (0.16) $\times 10^6\ M^{-1}$ at pH 5.0 to 2.18 (0.06) $\times 10^6\ M^{-1}$ at pH 9.4 at 25 °C, with a midpoint of pH 7.2. This pH profile of binding constants for T_4 binding to BSA resembles that reported for T_4 binding to HSA.[8,9] The increase of the binding constants for both albumins at higher pH values has been explained by the electrostatic interaction between the negative charge of the ionized phenolic hydroxyl group of T_4 ($pK_a=6.73$[8–10]) and the positively charged Lys412 of BSA and/or Lys414 of HSA.[1] However, contrary to the case of T_4 binding to BSA, no pH dependence of the binding constants could be observed in the case of T_4 binding to G-BSA. The binding constants for the T_4–G-BSA system are higher than those for the T_4–BSA system at pH < 7 and lower at pH > 7. This result suggests that the glycosylation of BSA changes the binding properties of T_4 to BSA to prevent the electrostatic interaction between T_4 and Lys412. In the case of G-HSA, the glycosylated amino acid residue was identified as Lys525,[2] and in the crystal structure[11] Lys525 was found to be located not far from the active Lys414 in T_4 binding to HSA.[1] At the present time, the glycosylated amino acid residue of G-BSA has not

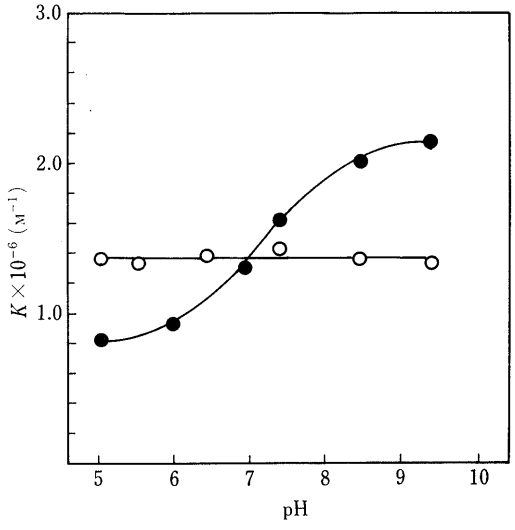

Fig. 1

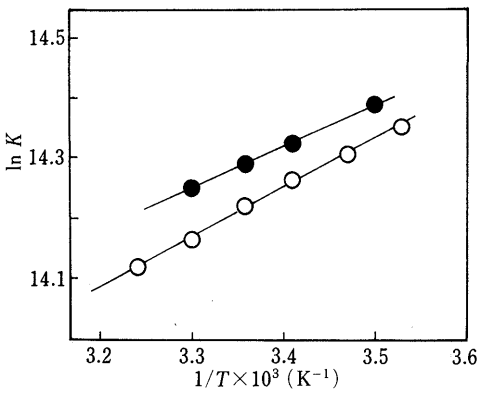

Fig. 2

yet been identified. However, based on the resemblance between the amino acid sequences around Lys521–Lys522 of BSA and Lys524–Lys525 of HSA,[1] the glycosylated residue would most probably be Lys521 and/or 522 in BSA, and thus they have an effect on the active Lys412[1] in T_4 binding so as to prevent the electrostatic interaction between T_4 and Lys412.

Figure 2 shows the Van't Hoff plot of the apparent binding constants for T_4 binding to G-BSA and BSA at pH 7.4. In both cases, $\ln K$ is linearly related to $1/T$ in the temperature range 10—35 °C. The thermodynamic parameters at pH 7.4 and 8.5 are summarized in Table I. The free energy, the enthalpy and the entropy changes of both G-BSA and BSA were almost the same, although small differences can be seen at both pH's. These results, especially the data for binding free energy indicate that although binding affinity of BSA to T_4 was only little affected by the glycosylation of BSA, its pH dependence was diminished, leading to the same conclusion as above; electrostatic interaction between T_4 and G-BSA was suppressed by the glycosylation of BSA.

TABLE I. Thermodynamic Parameters for T_4 Binding to Both Glycosylated and Intact BSA in 0.1 M Sodium Phosphate Buffer, pH 7.4 and 8.5 at 25 °C

	pH	ΔG (kcal mol^{-1})	ΔH (kcal mol^{-1})	ΔS (e.u.)
Glycosylated BSA	7.4	−8.38 (0.06)	−1.65	22.6
	8.5	−8.37 (0.03)	−1.92	21.6
BSA	7.4	−8.48 (0.06)	−1.37	23.9
	8.5	−8.56 (0.06)	−1.66	22.8

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

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