Effect of pH and Small Inorganic Ions on Binding of Sulfadimethoxine and Sulfaphenazole to Human Serum Albumin Measured by Circular Dichroism

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The binding of sulfadimethoxine and sulfaphenazole to human serum albumin (HSA) has been shown by circular dichroism measurements to be dependent on the N–B transition. The secondary drug binding sites were found to be optically active in the B conformation form in HSA but optically inactive in the N form. Moreover, the drug–HSA interaction in Tris–HCl buffer seems to be more sensitive to the conformational change in HSA, compared with that in the phosphate buffer.

Keywords human serum albumin; circular dichroism; microcalorimetry; conformational change; N–B transition; induced ellipticity; protein binding

Several authors have reported a pH-dependent conformational change to occur in serum albumin at around the pysiological pH.[1-3] Leonard *et al.*,[1] using optical rotation studies at 313 nm, firstly discovered a conformational change in bovine and human serum albumin (BSA and HSA). This conformational change is now commonly referred to as the N–B transition.[3] Histidine, whose imidazole residues have a pK_a of 6.4—7.0, seems to be involved in this transition. At physiological pH, albumin can exist in two forms, the N- and the B-form. Small inorganic ions such as chloride and calcium ions have been shown to affect the N–B transition.[3,4] The involvement of the N–B transition in drug–HSA interactions was firstly found with warfarin by using the circular dichroism (CD) technique.[4,5] Such CD studies can give information about the nature of the drug binding sites because the extrinsic Cotton effects generated by the drug–HSA interaction are sensitive to the binding environment on the albumin.

The binding of sulfa drugs including sulfadimethoxine (SDM) to albumins has been studied by several investigators.[6-9] However, they did not investigate the effect of the N–B transition on the binding of sulfa drugs to albumins. In the present work, we describe the effect of albumin conformation on the interactions of SDM and sulfaphenazole (SPZ) with HSA, determined by using a CD technique.

Experimental

Materials SDM and SPZ were supplied by Daiichi Pharmaceutical Co. (Tokyo) and Dainihon Pharmaceutical Co. (Osaka), respectively. HSA fraction V (lot No 84F-9399) was obtained from Sigma Chemical Co. (St. Louis, MO). All other materials were of reagent grade and all solutions were prepared in deionized and distilled water.

Apparatus and Methods CD measurements were made on a Jasco J-50A recording spectropolarimeter (Tokyo) using a 10 mm cell at 25 °C. Ultraviolet (UV) spectra were measured with a Hitachi 556S dual-wavelength spectrophotometer (Tokyo) using a pair of split-compartment, tandem-mixing cells. Microcalorimetric measurements were made on an LKB 2107-121 flow microcalorimeter (Bromma, Sweden). Dialysis experiments were performed using a Sanko Plastic dialysis cell (Fukuoka). After equilibration (12 h), the free concentration of the drug was assayed by high performance liquid chromatography (HPLC). The binding data obtained by CD, UV and microcalorimetry were treated according to previous reports.[10-12] Full details of the data treatment are given elsewhere.[10-12]

Results and Discussion

SDM showed a positive ellipticity near 255 nm and a negative ellipticity near 280 nm following the binding to HSA. A positive extrinsic Cotton effect was also generated near 285 nm for the SPZ–HSA system. Fortunately, these induced ellipticities were large enough to be quantitatively investigated.

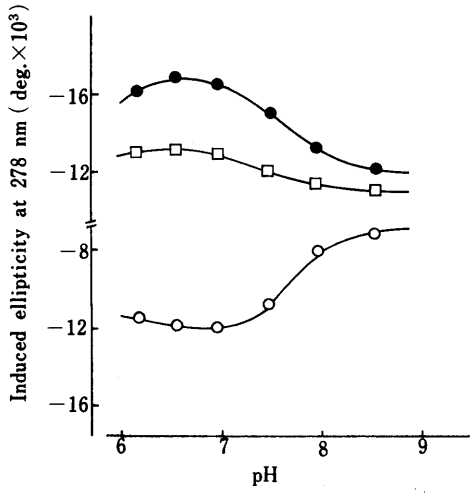

Fig. 1. Effect of pH on the Induced Ellipticities Arising the Binding of SDM and SPZ to HSA in Phosphate Buffer of pH 7.4 at 25 °C

—●—, DSM–HSA system at 254 nm; —○—, SDM–HSA system at 278 nm; —□—, SPZ–HSA system at 286 nm; [SDM]=[SPZ]=2×10^{-5} M; [HSA]=1×10^{-5} M.

TABLE I. Binding Parameters of SDM and SPZ to HSA at Various pH Values at 25 °C Obtained by the CD Method

pH	SDM			
	n_1	K_1 ($M^{-1}\times10^{-5}$)	n_2	K_2 ($M^{-1}\times10^{-4}$)
6.5	1.2±0.1	3.7±0.3		
7.4	1.2±0.1	3.0±0.5		
8.5	1.1±0.1	2.7±0.6	0.8±0.2	0.5±0.2

pH	SPZ			
	n_1	K_1 ($M^{-1}\times10^{-5}$)	n_2	K_2 ($M^{-1}\times10^{-4}$)
6.5	0.7±0.1	8.0±0.4		
7.4	0.7±0.1	7.5±0.4		
8.5	0.8±0.1	7.0±0.5	0.9±0.2	0.5±0.2

TABLE II. Binding Parameters of SDM to HSA at Various pH Values at 25 °C Obtained by the Dialysis Method

pH	n_1	K_1 ($M^{-1} \times 10^{-5}$)	n_2	K_2 ($M^{-1} \times 10^{-4}$)
6.5	1.3±0.1	0.7±0.2	1.7±0.3	0.4±0.2
7.4	1.3±0.2	0.9±0.2	1.9±0.3	0.6±0.2
8.5	1.1±0.2	0.7±0.3	1.2±0.4	0.4±0.2

TABLE III. Binding Parameters of SDM and SPZ to HSA at pH 7.4 and 25 °C

	SDM			
	n_1	K_1 ($M^{-1} \times 10^{-5}$)	n_2	K_2 ($M^{-1} \times 10^{-5}$)
CD	1.2±0.1	3.0±0.5		
UV	0.9±0.2	1.0±0.3		
Microcalorimetry	1.0±0.3	0.8±0.3		
Dialysis	1.3±0.2	0.9±0.2	1.9±0.3	0.6±0.2

	SPZ			
	n_1	K_1 ($M^{-1} \times 10^{-5}$)	n_2	K_2 ($M^{-1} \times 10^{-5}$)
CD	0.7±0.1	7.5±0.4		
UV	0.9±0.2	2.8±0.3		
Microcalorimetry	1.0±0.3	0.9±0.3		
Dialysis	1.3±0.2	1.8±0.3	1.9±0.3	0.6±0.2

The binding parameters and the induced ellipticities of the complexes of sulfa drugs with albumins are influenced by the pH of the solutions.[6,8] The effects of pH on the binding of SDM and SPZ to HSA were carefully re-examined in the current work. As shown in Fig. 1, the induced ellipticities of drug–HSA complexes decrease significantly on raising the pH. The pH profile of the ellipticities can not be explained by changes in the states of ionization of SDM and SPZ, because their pK_as have been reported to be 5.98 and 5.87, respectively.[13] Therefore, it is presumed that the pH dependence of the induced ellipticities for drug–HSA interactions can be ascribed to the N–B transition.[3-5] This hypothesis may be supported by the fact that the ellipticities around pH 8 are greater than those around pH 9 even though the drugs are fully ionized over pH 8.

Moreover, the effects of pH on the binding parameters of the SDM–HSA complex were examined. The Scatchard plots obtained by the dialysis method showed curvature over the pH region of these investigations and so the data were analyzed assuming two independent classes of binding sites by using the computer programs described by Goto *et al.*[14] However, the Scatchard plots of the CD data at pH 6.5 and 7.4 were linear, in contrast to those at pH 8.5. This apparently suggests that the ellipticities for the secondary site are enhanced following the change to the B-form. It should be noted that the ellipticities at the secondary drug binding site change from optically inactive to optically active type, depending upon the conformation change in the albumin. This is the first report of a dramatic involvement of the N–B transition in the ellipticities arising from drug–HSA interaction.

TABLE IV. Binding Parameters of SDM and SPZ to HSA in 0.1 M Phosphate and Tris–HCl Buffers of pH 7.4 and 25 °C Obtained by the CD Method

	SDM		SPZ	
	n_1	K_1 ($M^{-1} \times 10^{-5}$)	n_1	K_1 ($M^{-1} \times 10^{-5}$)
Phosphate	1.2±0.1	3.0±0.5	0.7±0.1	7.5±0.4
Tris–HCl	1.3±0.2	3.6±0.5	0.8±0.1	9.0±0.6

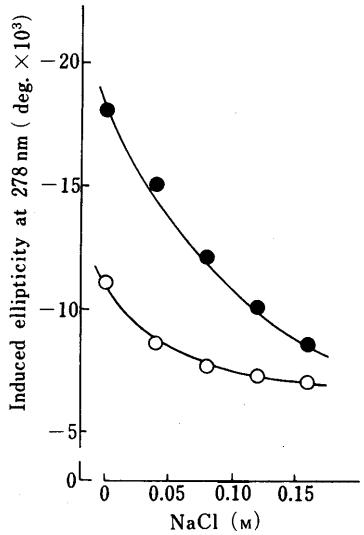

Fig. 2. Effect of Chloride Ions on the Induced Ellipticities for SDM–HSA Interaction in Phosphate and Tris–HCl Buffers of pH 7.4 at 25 °C
—○—, in phosphate buffer; —●—, in Tris–HCl buffer; [SDM] = 2×10^{-5} M; [HSA] = 1×10^{-5} M.

The primary binding constants for the complex decreased slightly or changed little with pH (Tables I and II). In addition, the secondary binding constants changed little with pH. Therefore, the pH profile in Fig. 1 can be explained in terms of a change in molecular ellipticity of the complex rather than a change of the binding constant.

At pH 6.5 and 7.4, the CD technique detects only the primary binding sites which generate the induced ellipticities, and similar results were obtained by other methods. As shown in Table III, only the primary binding constants were estimated, suggesting that spectroscopic and calorimetric techniques detect only the primary binding sites which generate spectral changes and evolve binding heat changes.

Futhermore, the effects of buffer composition and chloride ion on the binding of SDM to HSA were investigated. Although the binding parameters for the SDM–HSA interaction are the same in phosphate and Tris–HCl buffers (Table IV), interestingly the effects of chloride ion on the binding in the two buffers are different, as shown in Fig. 2. It is well-known that the N–B transition in the albumin is affected by chloride and calcium ions.[4,5] Therefore, the results in Fig. 2 may be due to the conformational change of HSA rather than displacement. That is, the chloride ions seem to affect the type of the interaction or the nature of the binding site by an indirect effect on both the N and B forms. The binding in Tris–HCl buffer is more affected by the

addition of chloride ions than that in phosphate buffer, implying that the drug–HSA interaction in Tris–HCl buffer is more sensitive to this conformational change in HSA, although the mechanism remains to be investigated in detail.

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