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Ibuprofenlysine Binding to Human and Bovine Serum Albumin Using a Fluorescence Probe Technique

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Abstract□The possibility of using a fluorescence probe technique for the study of ibuprofenlysine binding to human and bovine serum albumin was investigated. 1-anilino-8-naphalenesulfonate was used as the probe. The number of binding sites of human and bovine serum albumins for ibuprofenlysine appears to be 4 and 2, respectively. By using this technique, the association constants were found to be $1.533\times10^4M^{-1}$ and 2.238×10^4 M^{-1}, respectively.

Keywords□Ibuprofenlysine-binding to human and bovine serum albumins, fluorometric analysis; Fluorometry-study of binding of ibuprofenlysine to human and bovine serum albumin; Protein-drug binding-determination, fluorescent probe indicators.

Bloomfield *et al.*[1] reported that ibuprofen (300 or 900 mg orally) provided pain relief following episiotomy.

Matsumoto *et al.*[2] indicated that ibuprofen was effective in inhibiting adjuvant-induced arthritis in rats and the inhibiting effect was 3 times that of phenylbutazone and 5-10 times that of aspirin.

The inhibitory effect of ibuprofen on heat denaturation of bovine serum albumin(BSA) was similar to that of indomethacin and phenylbutazone but 10 times that of aspirin[1]. Matsumoto *et al.*[3] also reported that the efficacy of a group of antiinflammatory drugs, including a series of phenylalkanoic acids had activity which stabilized the erythrocyte membrane. Because the most potent of antiinflammatory drugs strongly stabilized blood serum albumin and had a high affinity for erythrocytes. Kalhen and Loyen[4] showed that antiinflammatory-antirheumatic drugs inhibited heat induced hemolysis of human erythrocytes. Order is as follows; cinnopentazone>niflumic acid> metiazinic acid > azapropazone > fenclozic acid>alclofenac>ibuprofen>ibufenac.

The binding of drugs to serum albumin has been known to effect the bioavailability and level of response of certain pharmaceuticals[5~7]. Indomethacin displaces ibuprofen bound to human serum albumin (HSA) in vitro and protein binding of indomethacin is decreased by the presence of ibuprofen[8]. Sudlow *et al.*[9] reported two binding site of ibuprofen bound to HSA by using probe, 5-dimethylaminonaphthalene sulfonamide or dansylsarcosine. Although ibuprofen is widely used as

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antiinflammatory-antirheumatic agents, this drug has poor solubility in water. The solubility of ibuprofen is increased markedly by the addition of lysine to the carboxyl group of ibuprofen.

The purpose of present work was to obtain the association constant and number of binding sites of ibuprofenlysine to human and bovine serum albumins using fluorescence probe technique.

EXPERIMENTAL

Materials

Ibuprofenlysine used was obtained from Il-Yang Pharm. Co. and recrystallized from benzene. Crystalline human serum albumin (Sigma Co.) and crystalline bovine serum albumin (Polyscience Inc.) were used. Their molecular weight was assumed to be 69000. The fluorescence probe, 1-anilinonaphthalene-8-sulfonate(ANS) was purchased from Sigma Co. All other chemicals used were of analytical reagent grade.

Instruments

All fluorescence measurements were made with Bairo-Automic Spectrophotometer Model FC 100 equipped with 150 watts xenon lamp and spectra were recorded on Bryans Model 2500 X-Y recorder. All fluorescence emission spectra in this study were uncorrected. Mechanical shaker (Dong-Yang Machine Co.) was used.

Methods

The binding of the probe, ANS, to human serum albumin(HSA) and bovine serum albumin(BSA) were determined by Brand *et al.*[12]. Their method was modified for present study as follows. HSA and BSA were dissolved in phosphate buffer (0.05M, pH 7.4). The probe, ANS, was dissolved in methanol at a concentration of 1×10^{-3}M. 3 ml of high concentration (7.25×10^{-6}M) and 3 ml of low concentration (7.25×10^{-7}M) of serum albumin were titrated by successive additions of 1 μl of ANS solution (1×10^{-3}M), using a microsyringe at room temperature.

Ibuprofenlysine was dissolved in low concentration of serum albumin solution to make the concentration of 5.673×10^{-4}M. Binding of Ibuprofenlysine to serum albumins (HSA, BSA) was determined by titrating 3 ml of mixture of ibuprofenlysine and serum albumin with successive additions of 1 μl of ANS solution.

The excitation and emission wavelengths for ANS were 386 and 468 nm, respectively. All measurements were performed in 1 cm quartz cells with 32 nm entrance and exit slits.

TREATMENT OF DATA

To calculate the fractions of bound(X) and free(1-X) ANS concentrations from fluorescence data, the following equation.[10] was applied.

$$X=\frac{I_o/I_f-1}{I_b/I_f-1} \qquad \cdots\cdots(1)$$

where

I_o=fluorescence intensity of low protein concentration

I_b=fluorescence intensity of high protein concentration

I_f=fluorescence intensity of solutions with-

out protein

After the value X was found for each point along the titration curve, Scatchard equation applied to calculate the probe-to-protein binding parameters.

$$\frac{\bar{V}}{A} = nKa - \bar{V}Ka \quad \cdots\cdots\cdots(2)$$

where

$\bar{V}$=number of moles of bound probe per mole of protein

A=concentration of free probe

n=number of binding sites on the protein molecule.

K_a=association constant of the probe to the protein.

The $\bar{V}$ is determined by multiplying the value for X by the ratio of the total probe concentration to the total protein concentration in solution. When $\bar{V}/A$ is plotted against $\bar{V}$, a straight line is obtained with a slope equal to Ka. The ordinate and abscissa intercepts of this line give nKa and n, respectively.

The association constant for ibuprofenlysine can be calculated according to the Klotz equation.[12]

$$K_b = \frac{nP_oK_a[A] - Ka[A][PA] - [PA]}{B_t K_a[A] - P_o K_a[A] + K_a[A] + \frac{}{K_a[A][PA] + [PA]} \times \frac{K_a[A]}{[PA]}} \quad \cdots\cdots\cdots(3)$$

where

K_b=association constant for competitor

K_a=asociation constant for probe

A =concentration of free probe

PA=concentration of bound probe

n =number of binding sites

P_o= low protein concentration

B_t=total concentration of ibuprofenlysine

RESULTS AND DISCUSSION

The use of fluorescence spectroscopy to study the binding of drug molecules to proteins has been well established.[13,14]

Figures 1-1 and 1-2 indicated the fluorescence emission spectra of ANS in the absence and presence of serum albumin (BSA, HSA) in pH 7.4 phosphate buffer (0.05M). The fluorescence intensity of ANS in pH 7.4 phosphate buffer is not significant (curve C), but in the presence of albumin, the fluorescence intensity of ANS is greatly enhanced(Curve A). However curve B shows that the fluorescence emission spectra of ANS is reduced by the addition of ibuprofenlysine. A decrease in fluorescence of ANS-serum

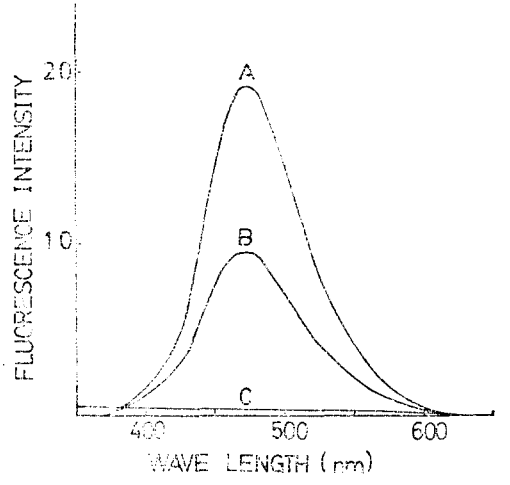

Fig. 1-1: Fluorescence emission spectra of the ANS-BSA complex in the presence(A) and in the absence(C) of bovine serum albumin (7.25×10^{-7}M) in pH 7.4 phosphate buffer. Curve B is the emission spectra of the ANS-BSA complex in the presence of ibuprofenlysine (5.673×10^{-4}M).

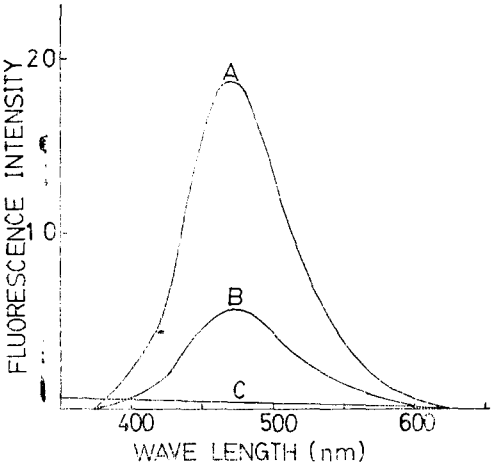

Fig. 1-2: Fluorescence emission spctra of the ANS-HSA complex in the presence(A) and in the absence(C) of human serum albumin (7.25×10^{-7}M) in pH 7.4 phosphate buffer. Curve B is the emission spectra of the ANS-HSA complex in the presence of ibuprofenlysine (5.673×10^{-4}M).

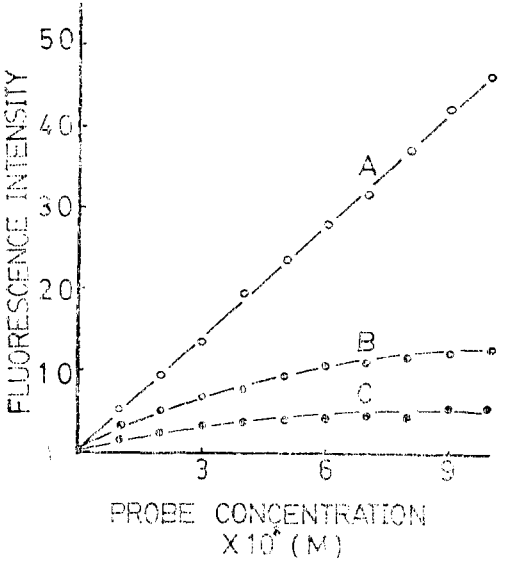

Fig. 2: Fluorescence titration curves of BSA with at higher(A: 7.25×10^{-6}M) and lower(B: 7.25×10^{-7}M) concentrations with ANS. Curve C is the titration curve of lower BSA concentration with ANS in the presence of ibuprofenlysine.

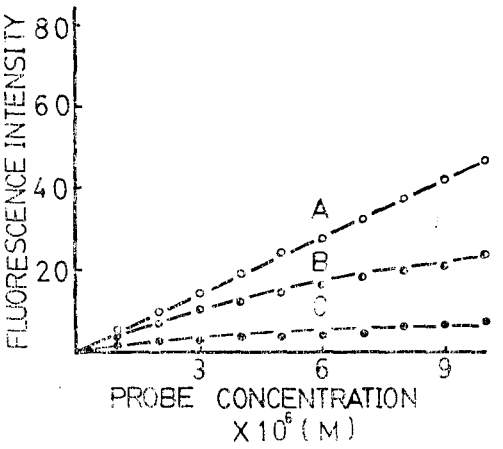

Fig. 3: Fluorescence titration curves of HSA at higher(A:7.25×10^{-6}M) and lower (B: 7.25×10^{-7}M) concentrations with ANS. Curve C is the titration curve of lower HSA concentration with ANS in the presence of ibuprofenlysine.

albumin complex in the presence of ibuprofenlysine is indication of the competition between ANS and drug for the binding sites on the protein.[8,14] Enhancement of the fluorescence of the ANS upon addition to BSA and HSA at two concentrations and the subsequent decrease of fluorescence in the presence of the binding competitor, ibuprofenlysine, was used to calculate the binding parameters for the probe and ibuprofenlysine.

The fluorometric titration results of BSA and HSA are shown in Fig. 2 and Fig. 3, respectively.

Curve A in both figures is linear when fluorescence is plotted versus increasing ANS concentration at high serum albumin concentration (7.25×20^{-6}M). This indicates that all ANS added is fully bound under

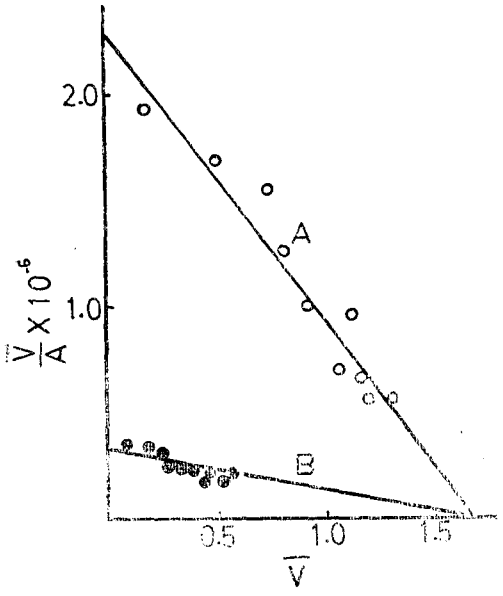

Fig. 4: Scatchard plots for ANS-BSA(A) and ibuprofenlysine-BSA(B) complex.

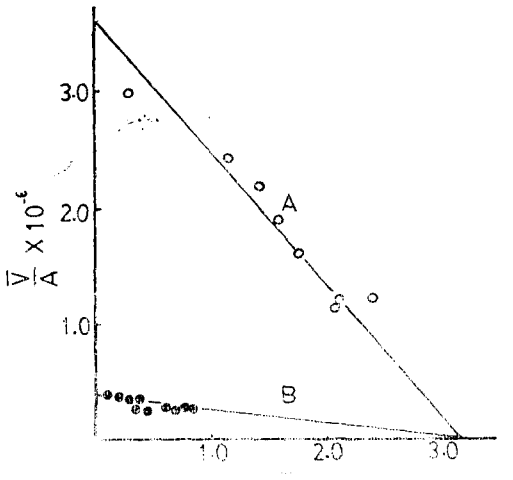

Fig. 5: Scatchard plots for ANS-HSA(A) and ibuprofenlysine-HSA(B) complex.

these conditions. Curve B in both figures shows that at low serum albumin concentration (7.25×10^{-7}M), the probe is only partially bound. Curve C in both figures is the titration curve for the probe at same low serum albumin concentration in the presence of ibuprofenlysine (5.673×10^{-4}M). A decrease in fluorescence of ANS-serum albumin complex is observed. When the drug is added to protein solution prior to titration, binding sites are initially occupied by drug molecules with subsequent competition for the binding sites between drug and probe molecules following titration with the probe.

Figure 4 shows the Scatchard plots for the BSA-ANS complex(line A) and for the BSA-ibuprofenlysine complex(line B) at the serum albumin concentration of 7.25×10^{-7}M. The intercepts on the abscissa are identical for the two compounds, but the slope is decreased in the presence of drug. This indicates a competition between the probe and drug for the binding sites measured.

Based on the graphs, the BSA molecule appeared to have 2 binding sites. The binding constant for ANS with BSA was found to be $1.79\times10^{6}M^{-1}$ and by means of Eq (3), the binding association constant for ibuprofenlysine with BSA was also estimated to be $2.234\times10^{4}M^{-1}$.

Figure 5 is the Scatchard plots for the HSA-ANS system and for the straight line with a common absissa intercept indicates the competitive nature of the binding sites for the HSA-ANS interactions were found to be $8.59\times10^{5}M^{-1}$ and 4, respectively. The association constant for ibuprofenlysine with HSA was calculated according to Eq(3) and found to be $1.53\times10^{4}M^{-1}$.

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