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Spectroscopic Techniques in the Study of Protein Binding: The Use of 1-Anilino-8-naphthalenesulphonate as a Fluorescent Probe for the Study of the Binding of Iophenoxic and Iopanoic Acids to Human Serum Albumin

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SUMMARY

SUDLOW, GILLIAN, BIRKETT, DONALD J., AND WADE, DENIS N.: Spectroscopic techniques in the study of protein binding: the use of 1-anilino-8-naphthalenesulphonate as a fluorescent probe for the study of the binding of iophenoxic and iopanoic acids to human serum albumin. *Mol. Pharmacol.* **9**, 649–657 (1973).

The binding of iopanoic and iophenoxic acids to human serum albumin (HSA) has been studied by spectroscopic techniques. The protein fluorescence of human serum albumin was quenched by the binding of either drug. Analysis of this quenching indicated that iophenoxic acid binds very tightly to at least one site on the albumin molecule so that, at less than a 1:1 ratio of iophenoxic acid to HSA, almost no drug exists free in solution. This provides an explanation for the unusual pharmacokinetics of this drug. The binding of 1-anilino-8-naphthalenesulphonate (ANS) to human serum albumin has been studied and is shown to be consistent with strong binding at one site and weaker binding at three further sites. The fluorescence of ANS bound to HSA was increased by iophenoxic acid and decreased by iopanoic acid. Measurements of fluorescence emission spectra and fluorescence lifetimes indicated that the drug-induced changes in fluorescence were due to changes in the quantum yield of the bound ANS. It is concluded that iophenoxic and iopanoic acids induce different changes in albumin conformation which can be detected by changes in the fluorescence of bound ANS. Iophenoxic acid enhanced the fluorescence of ANS bound to its tight site but displaced ANS from the weaker binding sites. Furthermore, 1 mole of iophenoxic acid displaced more than 1 mole of ANS, indicating that this effect was also related to the drug-nduced change in the albumin structure.

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INTRODUCTION

Iophenoxic acid (3-amino-α-ethyl-2,4,6-triiodohydrocinnamic acid) and iopanoic acid (3-hydroxy-α-ethyl-2,4,6-triiodohydrocinnamic acid) are two radioopaque contrast agents with similar structures but very different pharmacokinetic profiles. Iopanoic acid is eliminated from the body in a matter of weeks, whereas iophenoxic acid has a half-life of 2–3 years (1–3).

Mudge and his co-workers (4–7) carried out extensive studies on the metabolism and mode of excretion of these two drugs and found that the rates of hepatic conjugation and excretion of iophenoxic acid were critically dependent on the plasma con-

centration. At 100 μg/ml a 2-fold change in plasma level was associated with a 100-fold or greater change in conjugation rate (4). The uptake of both drugs into slices of renal cortex and liver was reduced by the addition of plasma proteins to the incubation medium, the effect being more marked with iophenoxic acid. Furthermore, the accumulation of both drugs in slices of liver and kidney cortex was rapidly reversed by the addition of plasma to the incubation medium (5, 6). These authors postulated that exceptionally strong protein binding might cause the unusual biological persistence of iophenoxic acid. They further suggested that the renal excretory and the hepatic metabolic processes are limited by the availability of free drug, this limitation being more marked with iophenoxic acid than with iopanoic acid (5, 6).

In the present study the binding of iopanoic and iophenoxic acids to human serum albumin has been studied by spectroscopic techniques. The intrinsic fluorescence associated with the tryptophan of albumin was found to be quenched when either drug was bound to the protein. Further study of this quenching indicated that iophenoxic acid was more tightly bound to albumin than was iopanoic acid.

1-Anilino-8-naphthalenesulphonate has been widely used as a probe for the structure of membranes and proteins (8). The binding of this probe to HSA has been studied and is shown to be consistent with strong binding to one site and weaker binding to three further sites.

Changes in the fluorescence of bound ANS[1] induced by the binding of iopanoic and iophenoxic acids to HSA indicated that the two drugs induce changes in the conformation of the protein. As the changes in ANS fluorescence on binding of the two drugs are different, it is concluded that different conformational states of the HSA molecule are induced. Evidence is presented that iophenoxic acid displaces ANS from its weaker albumin-binding sites and that this may be due to the change in the protein conformation.

[1] The abbreviation used is: ANS, 1-anilino-8-naphthalenesulphonate.

METHODS

Experimental Procedure

Solutions of electrophoretically pure crystalline human serum albumin (Hoechst Australia, Ltd.) were made up in sodium phosphate buffer (0.1 M, pH 7.4, with 0.9% NaCl), and the concentration was checked using the published extinction coefficient of $E^{1\%}_{1cm}$ = 5.3 at 280 nm (9). The free fatty acid content of this albumin as quoted by the manufacturers was 0.5 mEq/100 g of albumin. The magnesium salt of ANS (K & K Laboratories) was used without further purification. Solutions of ANS were made up in the above buffer at pH 7.4, and concentrations were checked using the published value of 4.95×10^3 for the molar absorption coefficient at 350 nm (10). Iopanoic and iophenoxic acids were a gift from Dr. G. H. Mudge (Dartmouth Medical School). The drugs were dissolved in a small amount of 0.01 N NaOH and diluted with buffer to the desired concentrations. The pH of the diluted solutions was 7.4.

Optical density was measured in a Unicam SP 3000 automatic ultraviolet spectrophotometer. Fluorescence measurements were made in a Perkin-Elmer MPF-3 spectrophotofluorometer. Fluorescence lifetimes were measured on a TRW 75A decay time fluorometer.

Titrations Using Tryptophan Fluorescence Quenching

Solutions of iopanoic and iophenoxic acids were made up in buffer containing HSA (3.7 μM). Aliquots (20 μl) were added to 2.0 ml of a solution containing HSA at the same concentration, and the protein fluorescence was measured at 340 nm, with excitation at 280 nm. Parallel titrations were performed using a solution of tryptophan made up to the same initial absorbance at the excitation wavelength as the HSA solution. The decrease in fluorescence of this control solution was used to correct for quenching due to the inner filter effect.

Binding of ANS to HSA

The use of fluorescence to measure binding characteristics in the HSA-ANS system required the following two steps.

Titration to measure limiting fluorescence. Titrations of ANS with HSA were used to establish the limiting fluorescence when all the ANS was bound. A 2.0-ml solution of HSA (196 μM) and ANS (2.8 μM) was successively diluted with a solution of ANS at the same concentration so that the ANS concentration remained constant while the HSA concentration decreased. After each dilution the ANS fluorescence was measured at 475 nm, with excitation at 400 nm.

The reverse titration was performed when it was desired to study the effects of low concentrations of HSA on ANS fluorescence. Aliquots (10 μl) of a solution containing 20 μM HSA and 2.25 μM ANS were added to a 2.0-ml solution of 2.25 μM ANS. After each addition the ANS fluorescence was measured at 475 nm, with excitation at 400 nm.

Titration of HSA with ANS. The limiting fluorescence (i.e., when ANS is completely bound) was then used to determine the amount of ANS bound and free at each point on a titration of HSA with ANS. Aliquots (20 μl) of a solution containing ANS (199 μM) and HSA (10 μM) were added to 2.0 ml of HSA at the same concentration, and the ANS fluorescence was measured. The fluorescence of ANS in buffer was negligible at these wavelengths.

Calculation of Results

The concentration of bound ANS in each solution was calculated using the equation

$$\mathrm{ANS}_b = \left(\frac{F_c}{F_b} \times 2.8\right) \mu\mathrm{M}$$

where ANS_b is the concentration of bound ANS, F_c is the observed fluorescence, and F_d is the fluorescence of a solution containing 2.8 μM ANS and 36 μM HSA, under which conditions the ANS is completely bound.

The concentration of free ANS (ANS_f) was then calculated by subtraction of the bound concentration from the total. Results were plotted according to the method of Scatchard (11), using the expression

$$\frac{r}{A} = nK_a - rK_a$$

where r is the number moles of ANS bound per mole of protein, n the number of binding sites, K_a the association constant, and A the concentration of free ANS. The plot of r/A against r was curved, and binding parameters were obtained by iteration in the equation

$$r = \frac{n_1 K_{a_1} A}{1 + K_{a_1} A} + \frac{n_2 K_{a_2} A}{1 + K_{a_2} A}$$

assuming integral numbers for n_1 and n_2.

RESULTS

Binding of Iophenoxic and Iopanoic Acids to HSA Studied by Tryptophan Fluorescence Quenching

The protein fluorescence of HSA was quenched by both iophenoxic and iopanoic acids and was almost abolished at high drug concentrations (Fig. 1). The protein fluorescence quenching varied directly with the concentration of iophenoxic acid until drug and protein were present in equimolar amounts, suggesting that at less than equimolar concentrations iophenoxic acid is stoichiometrically bound to HSA.

The variation in fluorescence quenching as a function of iopanoic acid concentration was curved over the whole range studied, indicating that this drug was bound more weakly by HSA (Fig. 1).

Binding of ANS to HSA

ANS had very low fluorescence in water but became highly fluorescent on binding to HSA, and the emission maximum was blue-shifted from 520 nm to 465 nm. The fluorescence of bound ANS reached a plateau at relatively low HSA concentrations (approximately 30 μM), so that no extrapolation was necessary to obtain the limiting fluorescence.

Binding studies using fluorescence enhancement depend on the assumption that the fluorescent quantum yield is the same for ANS bound at each HSA site. Several pieces of evidence suggest that this assumption is justified in the present case.

1. Mixtures of ANS and HSA excited at 280 nm emit light at 465 nm as a result of energy transfer from the protein to the bound dye. The fluorescence emission spectra

of solutions containing various ratios of HSA and ANS are shown in Fig. 2. As the ratio of ANS to HSA increased, there was a decrease in the protein fluorescence at 340 nm and a concurrent increase in the ANS fluorescence at 465 nm. There was an isoemissive point at 410 nm, suggesting that the quantum yield was constant up to a saturation of 3.7 moles of ANS per mole of protein (12, 13).

2. The fluorescence lifetime of ANS bound to HSA remained constant, at 14.8 nsec, up to a saturation of 3.7 moles of ANS per mole of HSA (Table 1).

3. The emission maximum is 465 nm at saturations of 0.2 and 3.7 moles of ANS per mole of albumin (Fig. 2).

The Scatchard plot for binding of ANS to HSA is shown in Fig. 3. The plot is nonlinear, indicating the presence of more

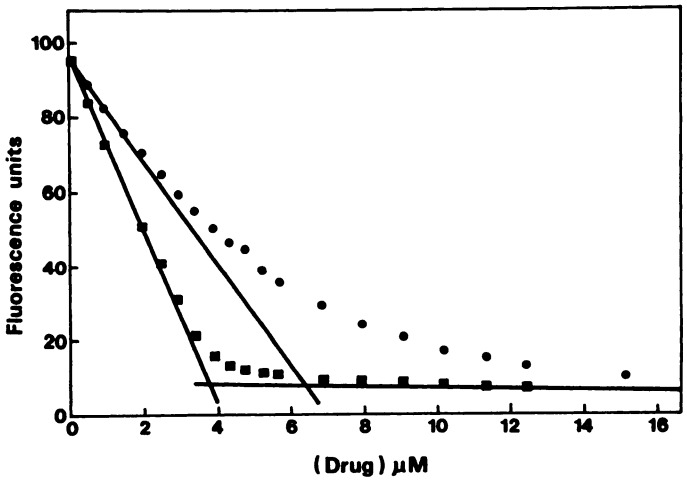

FIG. 1. *Quenching of albumin fluorescence by iopanoic and iophenoxic acids*
HSA (3.7 μM) was titrated with iopanoic acid (●——●) or iophenoxic acid (■——■). Fluorescence was measured at 340 nm, with excitation at 280 nm.

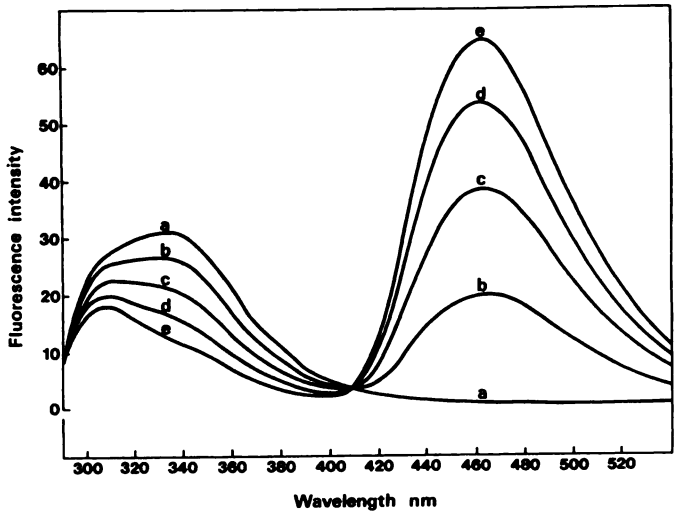

FIG. 2. *Fluorescence emission spectra of albumin-ANS mixtures*
Excitation was at 280 nm. Curves *a*, *b*, *c*, *d*, and *e* represent 0, 0.35, 0.7, 1.0 and 1.25 moles of ANS bound per mole of albumin, respectively. The isoemissive point was present up to a value of 3.7 moles of ANS bound per mole of HSA.

TABLE 1

Fluorescence lifetime measurements

Fluorescence lifetimes were measured in 0.1 M phosphate buffer, pH 7.4, with 0.9% NaCl at 25°. The values given are the means of eight measurements ± 1 SD.

Solution	Fluorescence lifetime
	nsec
Quinine sulphate (10 μM) in 0.1 N H_2SO_4	19.4 ± 1.05
HSA (43.5 μM) + ANS (2.5 μM)	14.8 ± 0.6
HSA (10.9 μM) + ANS (100 μM)	14.9 ± 0.4
HSA (43.5 μM) + ANS (2.5 μM) + iophenoxic acid (43.5 μM)	18.9 ± 0.5
HSA (43.5 μM) + ANS (2.5 μM) + iopanoic acid (43.5 μM)	13.0 ± 0.3
HSA (10.9 μM) + ANS (100 μM) + iophenoxic acid (10.9 μM)	15.4 ± 0.3

than one set of binding sites. The curve was fitted by iteration, assuming integral numbers for n_1 and n_2. A satisfactory fit was obtained with $n_1 = 1$, $n_2 = 3$, $K_{a_1} = 0.9 \times 10^6$ M^{-1}, and $K_{a_2} = 0.13 \times 10^6$ M^{-1}. The curve could not be fitted assuming $n_1 = 2$ and $n_2 = 2$.

Effects of Iophenoxic and Iopanoic Acids on Fluorescence of Bound ANS

Iopanoic and iophenoxic acid titrations. Addition of iophenoxic acid to a solution containing HSA and ANS with the HSA in excess, so that all the ANS was bound, caused an increase in the ANS fluorescence, which reached a maximum value of 122% at an iophenoxic acid to albumin ratio of 1:1 (Fig. 4). At greater iophenoxic acid to HSA ratios the ANS fluorescence began to fall slightly, until, at a 4:1 ratio of drug to HSA, it was approximately 80% of its original value.

Addition of iopanoic acid to a similar solution caused a steady, almost linear, decrease in the ANS fluorescence until, at a 4:1 ratio of drug to HSA, it was 40% of its original value.

Table 2 shows dialysis studies carried out to determine whether observed changes in fluorescence were due to changes in ANS binding. The conditions are comparable to those used in the fluorescence studies. The concentration of free ANS was 4% or less of the total in the presence or absence of drugs.

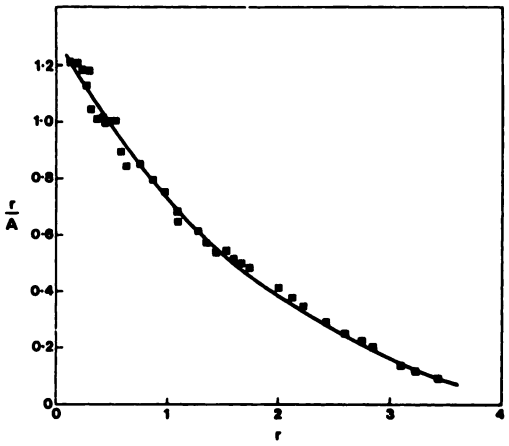

FIG. 3. *Scatchard plot for ANS binding to albumin*

HSA (10 μM) was titrated with ANS, and the fluorescence was measured (excitation at 400 nm, emission at 465 nm). The ANS concentration range was 1–100 μM. The concentrations of free and bound ANS were calculated from the fluorescence as described in the text. The points represent experimental values, and the solid line was computed using $n_1 = 1$, $n_2 = 3$, $K_{a_1} = 0.9 \times 10^6$ M^{-1}, and $K_{a_2} = 0.13 \times 10^6$ M^{-1}.

Albumin titrations. Limiting fluorescence titrations were performed, maintaining the ratio of iophenoxic acid to HSA at 1:1 and increasing the concentrations of both. As iophenoxic acid is very tightly bound (Fig. 1), this is equivalent to a titration of ANS with a complex of iophenoxic acid–HSA. The ANS fluorescence rose to a limiting value at about 30 μM iophenoxic acid–HSA, and this limiting fluorescence value was 25% greater than in the absence of drug (Fig. 5a). Furthermore, the emission maximum was slightly blue-shifted in the presence of drug from 465 nm to 460 nm, and the fluorescence lifetime rose from 14.8 to 18.9 nsec, an increase of 28%.

It can be seen, however, that at low HSA concentrations (i.e., high ANS:HSA ratios) the ANS fluorescence was decreased in the presence of iophenoxic acid (Fig. 5b). The point at which the fluorescence was the same with and without the drug occurred when ANS and HSA were present in about

a 1:1 molar ratio. This was true over a 4-fold range of ANS and HSA concentration.

Similar titrations were performed with ANS at a fixed concentration and increasing concentrations of HSA and iopanoic acid (maintained at a 1:1 or 1:2 ratio). In this case it could not be assumed that the drug was bound to one or two distinct sites on HSA. An equilibrium situation probably existed, with iopanoic acid distributed among several sites. Nevertheless these titrations did act as a comparison with that in the presence of iophenoxic acid.

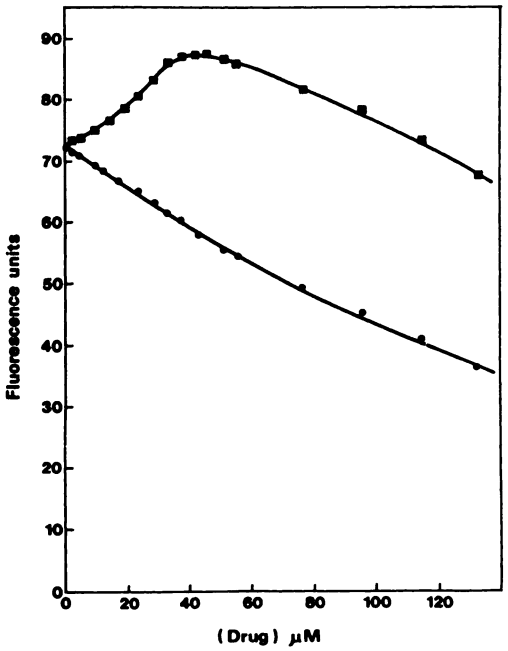

FIG. 4. *Effect of iopanoic and iophenoxic acids on fluorescence of ANS bound to HSA*

A solution containing HSA (41 μM) and ANS (2.5 μM) was titrated with iophenoxic or iopanoic acid. Changes in the concentration of HSA and ANS were not significant. Fluorescence was corrected for dilution. Excitation was at 400 nm, and the fluorescence was read at 465 nm. ■——■, iophenoxic acid; ●——●, iopanoic acid.

The limiting fluorescence values were 88% (1:1 ratio) and 80% (1:2 ratio) of that obtained with HSA alone. In each case the ANS fluorescence was lower than the control value over the whole range of HSA concentration. The ANS fluorescence lifetime in the presence of a 1:1 ratio of iopanoic acid fell from 14.8 to 13 nsecs, a decrease to 88% of the original value (Table 1).

ANS titrations. Figure 6 shows titrations of HSA and of the HSA–iophenoxic acid complex (1:1) with ANS. At an ANS concentration of 85 μM the fluorescence in the absence of drug reached a value corresponding to 3.4 moles of ANS bound per mole of albumin. In the presence of drug the fluorescence was 40% less than the control value and represented 2 moles of ANS bound per mole of HSA, assuming no change in quantum yield. The fluorescence lifetime of bound ANS was slightly increased, from 14.8 to 15.4 nsec, in the presence of iophenoxic acid (Table 1), indicating that the quantum yield was slightly increased. This suggests that the value of 2 moles of ANS bound per mole of albumin represents the upper limit for this figure and that 1 mole of iophenoxic acid

TABLE 2

Equilibrium dialysis studies

Studies were carried out in equilibrium dialysis cells (Chemical Rubber Company) which contained a volume of 1 ml on each side of the membrane. Solutions were allowed to equilibrate for 16 hr at 22°. The concentrations of ANS on sides 1 and 2 of the membrane were measured by diluting an aliquot with an equal volume of HSA (40 μM and 80 μM, respectively) and measuring the fluorescence. The lower limit of this method was 0.1 μM ANS, and standard curves were linear up to 30 μM ANS.

Before equilibration		After equilibration		ANS displacement
Side 1	Side 2	Side 1	Side 2	
				%
HSA (40 μM) + ANS (2.5 μM)	Buffer	ANS (2.5 μM)	ANS (<0.1 μM)	<4
HSA (40 μM) + ANS (2.5 μM) + iopanoic acid (40 μM)	Buffer	ANS (2.5 μM)	ANS (<0.1 μM)	<4
HSA (40 μM) + ANS (2.5 μM) + iophenoxic acid (40 μM)	Buffer	ANS (2.6 μM)	ANS (0.1 μM)	4

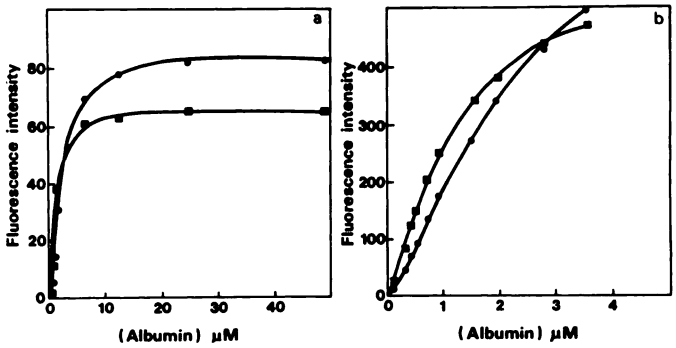

FIG. 5. *Effect of iophenoxic acid on fluorescence of ANS bound to albumin*

a. Limiting fluorescence titration. ANS (2.8 μM) was titrated with HSA or with HSA and iophenoxic acid maintained at a 1:1 ratio. ■——■, HSA alone; ●——●, HSA and iophenoxic acid.

b. Effect at low ratios of ANS to HSA. ANS (2.25 μM) was titrated with HSA alone (■——■) or with HSA and iophenoxic acid maintained at a 1:1 ratio (●——●). Excitation was at 400 nm, and fluorescence was read at 465 nm.

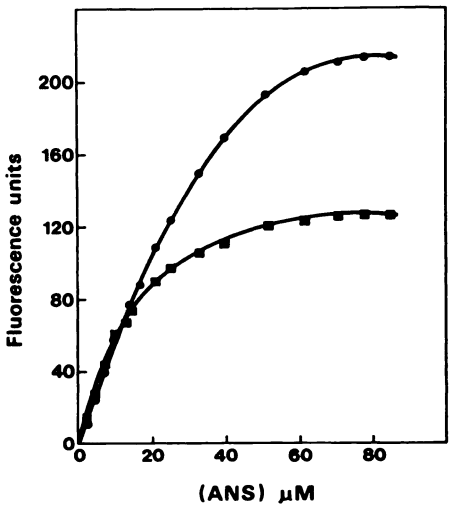

FIG. 6. *Effect of iophenoxic acid on titration of albumin with ANS*

HSA (10 μM) was titrated with ANS, and the fluorescence was measured at 465 nm with excitation at 400 nm. ●——●, no iophenoxic acid; ■——■, iophenoxic acid (10 μM).

displaced 1.4 or more moles of ANS. A 2:1 ratio of iophenoxic acid to HSA resulted in only a 10% further decrease in ANS fluorescence. Dialysis experiments were carried out with 10 μM albumin and 80 μM ANS. Under these conditions additions of 10 μM iophenoxic acid displaced 14 μM ANS from the albumin, thus confirming that about 1.4 moles of ANS were displaced per mole of iophenoxic acid added.

At low ANS:HSA ratios the fluorescence in the presence of iophenoxic acid was higher than in its absence. The crossover point occurred at about equimolar concentrations of ANS and HSA. This is in close agreement with the reverse titration shown in Fig. 5b.

Titrations of HSA and iopanoic acid (1:1 and 1:2 ratios) with ANS were also performed. In each case the ANS fluorescence was lower than in the absence of drug over the whole ANS concentration range. The limiting values were 14% and 24% less, respectively, than the control value.

DISCUSSION

The spectroscopic techniques used in this study demonstrate significant differences in the binding of iophenoxic and iopanoic acids to HSA. These differences help to explain the marked dissimilarity of pharmacokinetic profiles of the two drugs (1–3).

Iophenoxic and iopanoic acids almost completely quench the protein fluorescence of HSA. Both drugs exhibit appreciable absorbance at wavelengths longer than 300 nm (iophenoxic acid, λ_{max} 320 nm, $\epsilon = 6.25 \times 10^4$; iopanoic acid λ_{max} 311 nm, $\epsilon = 3.27 \times 10^3$). It is therefore likely that the quenching of the tryptophan fluorescence is due to dipole-dipole energy transfer from the tryptophan to the bound drug.

The degree of fluorescence quenching by iophenoxic acid is linearly related to the amount of drug added, up to an equimolar concentration with albumin (Fig. 1). This

provides strong evidence that iophenoxic acid binds very tightly to at least one site on the HSA molecule. The maximum enhancement of the fluorescence of the ANS-HSA complex at a 1:1 ratio of drug to protein (Fig. 4) supports this conclusion.

The effect of iopanoic acid on the protein fluorescence is quite different, in that the plot of fluorescence against concentration of drug is curved (Fig. 1). It is difficult to derive the binding parameters from the fluorescence quenching titrations, as binding at different sites may cause varying degrees of quenching. Even in the extreme case when quenching occurs as a result of binding at only one site, it may not be possible to derive the dissociation constant from such a titration. Binding at other sites on the protein may result in competition for the available drug, and thus a higher apparent dissociation constant will be derived. Price and Radda (14) have considered this case in some detail in relation to the binding of NAD^+ to glyceraldehyde 3-phosphate dehydrogenase.

The unusual pharmacokinetics of iophenoxic acid is likely to be a consequence of the very tight protein binding of this compound. At less than a 1:1 ratio of iophenoxic acid to HSA, almost no free drug would be available for either metabolism or excretion. This would explain the critical dependence of rates of excretion and hepatic conjugation on plasma levels, as well as the marked effect of plasma proteins on the tissue slice accumulation of iophenoxic acid (4–7).

The fluorescent probe ANS has been used to investigate further the interactions of iophenoxic and iopanoic acids with human serum albumin. Weber and his colleagues (15, 16) have shown that ANS binds to five sites on bovine serum albumin and have demonstrated cooperative interactions between sites. ANS binds tightly to HSA with a large fluorescence enhancement (about 300-fold) when compared with ANS in buffer. This enhancement is accompanied by a 55 nm blue shift in the emission maximum, from 520 nm in buffer to 465 nm when bound to HSA. The fluorescence lifetime of the bound ANS is 14.8 nsec. These features of the bound ANS fluorescence suggest that the dye is situated in a hydrophobic area of the protein with limited access of solvent to the dye and/or limited mobility of solvent molecules around the ANS binding site (17).

Several pieces of evidence have been presented to show that the quantum yield of fluorescence of ANS bound to HSA is constant up to a saturation of 3.7 moles/mole.

It is therefore possible to use fluorescence measurements to study the binding of ANS to HSA. The Scatchard plot is curved and was fitted by assuming one tight and three weaker binding sites. Further evidence for the presence of one tight ANS binding site is provided by the crossover points at equimolar concentrations of HSA and ANS in Fig. 5b and Fig. 6.

The binding of iophenoxic and iopanoic acids to the ANS-HSA complex results in changes in the ANS fluorescence which are considered strong evidence for conformational changes in the albumin molecule.

An increase in fluorescence is observed when iophenoxic acid is added to a solution of ANS and HSA, in which the ANS is fully bound (Fig. 4). The fluorescence reaches a maximum of 122% when iophenoxic acid is present in an equimolar concentration with HSA. Several points suggest that the increase in fluorescence is due to an increase in the quantum yield of ANS bound to its tight site. (a) Dialysis studies have shown less than 4% ANS free initially. (b) The enhancement is unlikely to be due to displacement of ANS to alternative sites with higher quantum yield, as there is no difference in the quantum yield of the various sites normally available for ANS binding. (c) There is a shift in the emission maximum from 465 nm to 460 nm. (d) The fluorescence lifetime is increased by 28%, from 14.8 nsec to 18.9 nsec. (e) An enhancement is seen only when ANS is present in less than equimolar concentrations with HSA (Fig. 5a and b and Fig. 6).

It is therefore likely that the very tight binding of iophenoxic acid to one site per mole of HSA results in a change in the albumin structure. This can be detected as a change in the quantum yield of ANS bound to its tight site. Furthermore, the results presented in Fig. 6 suggest that binding of

1 mole of iophenoxic acid can displace more than 1 equivalent of ANS from its weaker binding sites. The effect is presumably related to the drug-induced conformational change.

Iopanoic acid causes a decrease in the fluorescence of ANS bound to HSA (Fig. 4). This is likely to be due to a decrease in the quantum yield of ANS bound to its tight site. Limiting fluorescence studies indicate that there is no displacement of ANS from the protein, up to a 1:1 ratio of drug to HSA. Dialysis studies (Table 2) confirm that the observed 12% decrease in fluorescence is not due entirely to ANS displacement. Furthermore, fluorescence lifetime studies show a small (12%) but significant decrease in the lifetime of ANS bound to HSA at a 1:1 ratio of iopanoic acid to HSA. The decrease in quantum yield of ANS bound to HSA is probably due to a change in the protein structure induced by iopanoic acid binding. If this interpretation is correct, it means that iophenoxic and iopanoic acids induce different changes in the protein conformation when they bind.

Changes in the structure of albumins have been shown as a result of pH changes (18, 19) and of binding of detergents (20, 21) and anionic azo dyes (22). Attallah and Lata (23) proposed, on the basis of experiments with quenching of protein fluorescence that some steroids perturb the confirmation of albumin. Weder and Bickel (24) showed that desipramine binding to bovine serum album is cooperative in character, and they presented some spectral evidence that this was a result of changes in the albumin structure. It is shown in this paper that iophenoxic and probably iopanoic acid induce changes in the structure of HSA. It has further been shown that such structural changes may result in displacement of molecules bound at different sites on the albumin molecule. This suggests that drug interactions at the level of protein binding need not be considered strictly in terms of competitive displacement, but may also result from conformationally mediated interactions between distinct binding sites.

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