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5-(Dimethylamino)naphthalene-1-sulfonic Acid, a Fluorescent Probe of the Medium Chain Fatty Acid Binding Site of Serum Albumin†

Michael C. Doody,‡ Antonio M. Gotto, Jr., and Louis C. Smith*

ABSTRACT: Reversible binding of 5-(dimethylamino)-naphthalene-1-sulfonic acid (DNS) to human and bovine serum albumin has been monitored by changes in fluorescence intensity, wavelength maxima, and polarization. DNS has only one major binding site ($k_a = 5 \times 10^6$) and one minor site ($k_a = 3 \times 10^5$) on these proteins. The probe is competitively displaced from its high-affinity site by medium chain fatty acids and by *N*-acetyl-L-tryptophan. The binding site for DNS is highly hydrophobic and is considerably less polar than the hydrocarbon region of lipid bilayers. Resonance energy transfer indicates that the binding site is located 20.7 ± 2 Å from the single tryptophan residue of human serum albumin.

Serum albumin is the intravascular transport protein for cytotoxic lipolytic products, such as fatty acid, lysolecithin, and monoacylglycerol, as well as for many pharmacological agents and metabolites (Switzer & Eder, 1965; Arvidson & Belfrage, 1969; Sollenne & Means, 1979). The specificity of binding of fatty acids to albumin has been studied extensively (Tanford, 1980). The model that best accounts for experimental binding data has been described by Spector and colleagues (Fletcher et al., 1970; Spector et al., 1971; Ashbrook et al., 1972). According to this model, the binding of any fatty acid species to albumin occurs by a stepwise process at multiple individual binding sites that have progressively lower affinity with increasing molar amounts of fatty acid. Furthermore, the affinity of albumin for fatty acids decreases as the chain length decreases. A concise summary of previous studies has been prepared by Tanford (1980).

The interpretation of fatty acid binding data utilizing this model raises several interesting points. It is known that the decrease in affinity for the binding sites is complex with respect to alkyl chain length. A sigmoidal relationship exists between the logarithms of the first two stepwise affinity constants and chain length; however, similar plots of the stepwise binding constants for the sites of lower affinity do not exhibit this behavior. In addition, the affinity of albumin for fatty acids cannot be fully predicted on the basis of chain length alone, as would be expected from hydrophobic partitioning (Karush, 1954). Competition of octanoate acid with either *cis*-octadec-9-enoate or hexadecanoate to albumin shows apparent nonideal behavior (Ashbrook et al., 1972). Binding of the first mole of octanoate is not significantly affected by the long chain acids when the molar ratio of free fatty acid to albumin is less than 2. However, the binding of octanoate decreases substantially in the presence of high ratios of long chain fatty acid to albumin. Double-reciprocal plots of these data are nonlinear and do not have points of intersection in common for the binding isotherm of octanoate at different concentrations of hexadecanoate and *cis*-octadec-9-enoate (Ashbrook et al., 1972). By contrast, a double-reciprocal plot of the displacement of hexadecanoate by octanoate is linear and passes through zero (Meisner & Neet, 1978). The nonideal behavior in this system has been attributed to differences in configurational adaptability of the albumin molecule to fatty acids with different chain lengths (Spector, 1975) and to possible competitive allosterism (Meisner & Neet, 1978).

An alternate explanation for these observations is that albumin has one or more high-affinity sites with specificity for the medium chain fatty acids. Data that support this hypothesis have appeared (Cunningham et al., 1975; Doody & Smith, 1978; Koh & Means, 1979; Lee & McMenamy, 1980; Santos & Spector, 1974; Soltys & Hsia, 1978a,b). In a report of spin-label displacement studies, Soltys and Hsia identified three low-affinity bilirubin binding sites on human albumin that bind the spin probe and medium chain fatty acids, but not long chain fatty acids. No data on the relative binding affinities of these sites for individual fatty acids were reported. Santos & Spector (1974) have noted qualitative differences in the abilities of fatty acids of different chain length to perturb the fluorescence spectrum of 8-anilino-1-naphthalenesulfonic acid (ANS)[1] bound to multiple sites on human serum albumin. Cunningham et al. (1975) noted an effect of chain length on displacement of tryptophan from its binding site on albumin

† From the Division of Atherosclerosis and Lipoprotein Research, Departments of Medicine and Biochemistry, Baylor College of Medicine and The Methodist Hospital, Houston, Texas 77030. *Received July 24, 1981*. This work was supported by The Robert A. Welch Foundation Grant Q-343, by the National Heart and Blood Vessel Research and Demonstration Center, Baylor College of Medicine, a grant-supported research project of the National Heart, Lung and Blood Institute (HL 17269), and by U.S. Public Health Service Grant HL-15648.
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[1] Abbreviations: ANS, 8-anilino-1-naphthalenesulfonic acid; BSA, bovine serum albumin; HSA, human serum albumin; DNS, 5-(dimethylamino)naphthalene-1-sulfonic acid; Q_0, fluorescence quantum yield; $\bar{r}$, ratio of the molar concentrations of the bound ligand and of albumin.

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and suggested that the short chain fatty acids and indole bind at secondary long chain fatty acid sites. Koh & Means (1979) found that short chain fatty acids inhibit the interaction of *p*-nitrophenyl acetate with HSA. They concluded that there is a high-affinity binding site with dimensions that could accommodate decanoate and shorter fatty acids. Fatty acids with acyl chains longer than dodecanoate were not included in the study. Lee & McMenamy (1980), however, found that *N*-bromoacetyldecylamine blocks covalently the primary binding site for hexanoate, decanoate, and *N*-acetyl-L-tryptophan. Moreover, analysis of specific modified peptide fragments showed that this reagent did not react with the protein in the same region as *N*-bromoacetyltetradecylamine. In this report, we present evidence that a fluorescent ligand, 5-(dimethylamino)naphthalene-1-sulfonic acid (DNS), binds reversibly to a unique binding site for medium chain fatty acids on both bovine and huamn albumin. Longer chain fatty acids appear to be also capable of binding to this site, but with much lower affinity. Furthermore, *N*-acetyl-L-tryptophan binds at this site. The fluorescence properties of DNS also yield information on the physicochemical nature and location of this binding area for medium chain fatty acids.

Materials and Methods

Fraction V HSA (Sigma) and BSA (Armour) were delipidated by the charcoal treatment method of Chen (1967). Monomeric albumins were prepared by gel chromatography over Ultrogel ACA35. The buffer in all experiments was 18 mM Na_2HPO_4 and 1 mM sodium citrate, pH 7.4, containing 100 mM NaCl and 0.01% NaN_3. The separations were similar to those previously reported by Morrisett et al. (1975). Fractions containing monomer protein were pooled, stored at 4 °C, and used within 1 month. Protein concentrations were determined by reference values at 279 nm, based on an absorbance of 0.55 mg^{-1} mL^{-1} for HSA and 0.66 mg^{-1} mL^{-1} for BSA at this wavelength (Chen, 1967).

Fatty acids (Aldrich, gold label) had no visible impurities by thin-layer chromatography on silica in hexane–ethyl acetate (8:2 v/v), as visualized by either iodine vapor or ANS fluorescence (Gitler, 1972). When appropriate, aqueous stock solutions of fatty acids were heated to clarity before preparation of the fatty acid–albumin complexes at room temperature. DNS (Sigma) was sufficiently pure by thin-layer chromatography on silica in chloroform–methanol (7.5:2.5 v/v) to be used as received. The sodium salt was prepared by titration of an ethanolic solution with 1 N NaOH. DNS–albumin complexes were prepared with aliquots of a 1 mM stock solution of NaDNS. *N*-Acetyl-L-tryptophan (Sigma) was used as received and was added as aliquots of a 1 mM solution in ethanol. All solvents used were the highest grade commerically available and were stored over Linde 4-Å molecular sieves prior to use.

Fluorescence spectra were obtained on a Farrand Mark I dual monochromator fluorometer and recorded on a Houston Instruments Omnigraphic X-Y recorder. Spectra were uncorrected for photomultiplier tube response except where noted. Solutions were allowed to equilibrate at the temperatures indicated in the figures for 3–5 min after the addition of aliquots of either fatty acid or DNS. Fluorescence polarization measurements were made with an SLM Industries Model 4000 polarization/phase modulation instrument interfaced with Hewlett-Packard Model 10 desktop calculator. With the excitation at 350 nm, Corning 3-72 filters were used to isolate the emitted light at wavelengths greater than 390 nm. The temperature was maintained ±0.5 °C with a Lauda circulating bath. Polarization (p) is defined as

$$p = \frac{I_\parallel - I_\perp}{I_\parallel + I_\perp}$$

where $I_\parallel$ is the intensity of the emitted light polarized parallel to the plane of the excitation light and $I_\perp$ is the intensity of the emitted light polarized prependicular to the plane of the excitation light. All values were used on five determinations.

The value for energy transfer between the single tryptophan in HSA and DNS was calculated according to

$$R_0 = (9.79 \times 10^3 \text{ Å})(JQ_0k^2/n^4)^{1/6}$$

where R_0 is the distance at which the resonance energy transfer between a donor and acceptor is half the maximal value, Q_0 is the quantum yield of the donor in the absence of acceptor, n is the refractive index of the medium, J is the integral of the spectral overlap between donor emission and acceptor absorption, and k^2 is an orientation parameter (Sklar et al., 1980). For HSA, the Q_0 value was 0.3, obtained by comparison of the integrated corrected fluorescence spectra of HSA with that of an isoabsorbing solution of aqueous tryptophan, for which the quantum yield is known (Weber, 1953). The refractive index of the solution was assumed to be that of water. The spectral overlap integral was calculated from

$$J = \int_0^\infty \frac{f(\lambda)\epsilon(\lambda)}{\lambda^4}\, d\lambda$$

where $f(\lambda)d\lambda$ is the fraction of total donor intensity between λ and $\lambda + d\lambda$ and $\epsilon(\lambda)$ is the average extinction coefficient of the donor between λ and $\lambda + d\lambda$. The extinction coefficients in standard buffer were determined experimentally in a Cary 15 double-beam spectrophotometer. The sample contained 0.1 mM HSA and 0.1 mM DNS. The reference solution was 0.1 mM HSA. J was calculated to be equal to 3.1×10^{-15} cm M^{-1}. A value of 0.66 was assigned to k^2. Haas et al. (1978) have demonstrated by mathematical modeling that a very high degree of confidence can be placed in this value when either donor or acceptor, or both, has more than one transition dipole moment. They also determined values for three transition dipoles in the chromophore of DNS. Tryptophan is known to have two transition dipoles at wide angles to one another (Valeur & Weber, 1977). The calculated distances, under these conditions, are accurate to within 10%. R_0 was calculated to be 23.6 Å.

Results

Preliminary screening of many fluorescent probes that could be displaced from serum albumin by fatty acids showed that DNS was ideally suited for these experiments because of its water solubility and the magnitude of the changes in fluorescence behavior associated with binding to albumin. The fluorescence spectrum of DNS was remarkably different when the probe was bound to albumin and when it was in solution (Figure 1A,B). When the probe binds, the fluorescence maximum of DNS at 503 nm in aqueous solution shifted to shorter wavelength, which was 436 nm when bound to BSA and 433 nm when bound to HSA. In addition, when the probe was bound to albumin, the overall fluorescence quantum yield increased 3.8-fold for HSA and 3.6-fold for BSA, determined by integration of the uncorrected spectra in the presence of a large excess of protein. No further spectroscopic changes were noted after incubation at 37 °C for 1 day.

Addition of fatty acid to the DNS–albumin complexes produced changes in the spectra indicating displacement of DNS into the aqueous solution (Figure 1). The perturbation of DNS fluorescence induced by the addition of increasing

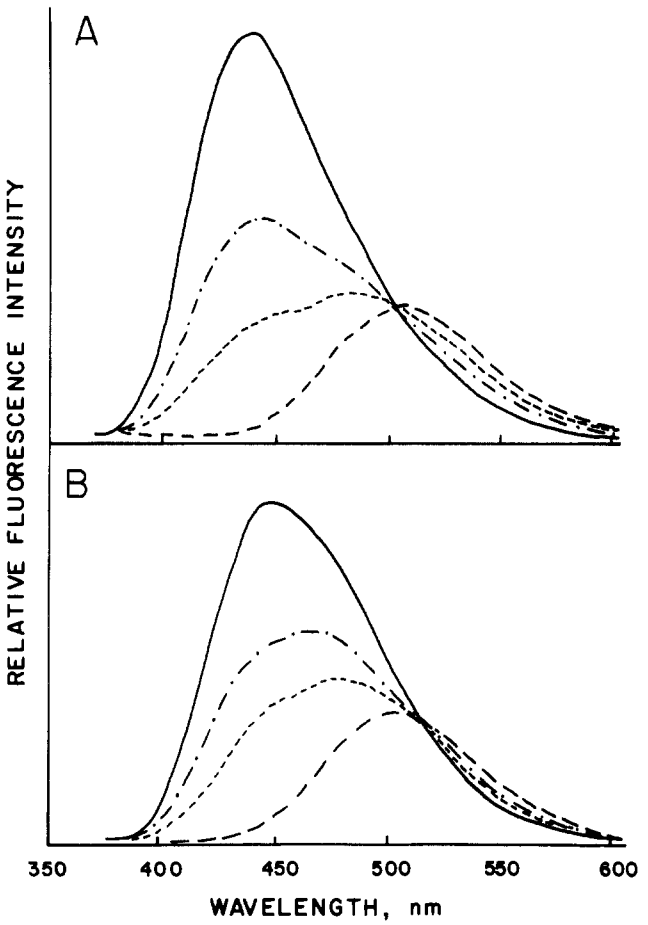

FIGURE 1: Fluorescence spectra of DNS bound to HSA (A) and BSA (B): 1 μM DNS in buffer (——); 1 μM DNS and 1 μM albumin (—); 1 μM DNS, 1 μM albumin, and 1 μM dodecanoate (---); 1 μM DNS, 1 μM albumin, and 2 μM dodecanoate (- - -). After the addition of aliquots of 0.4 mM fatty acid in buffer to the final concentrations as indicated, spectra were recorded 3–5 min later at 21 °C. The excitatioin wavelength was 325 nm.

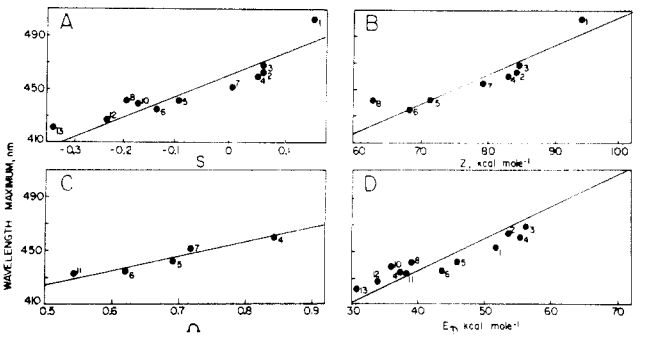

FIGURE 2: Wavelength maxima of DNS as a function of solvent polarity. Spectra of 1 μM solutions of NaDNS were recorded in duplicate at 25 °C. The excitation wavelength was 325 nm. Monochromator slits were 2 mm. Solvents were H_2O (1), 80% ethanol (2), ethylene glycol (3), methanol (4), acetonitrile (5), dimethylformamide (6), ethanol (7), chloroform (8), tetrahydrofuran (9), 1,4-dioxane (10), 1,2-dimethoxyethane (11), toluene (12), and hexane (13).

amounts of dodecanoic acid produced a progressive overall decrease in fluorescence intensity and a red shift to longer wavelengths. Isoemissive points were observed in the titrations of both HSA and BSA over the entire range of added fatty acid concentrations. At high fatty acid concentrations ($\bar{\nu} >$ 6), the spectrum of DNS reverted to one that corresponded to the relative intensity and fluorescence maximum of the probe in aqueous solution.

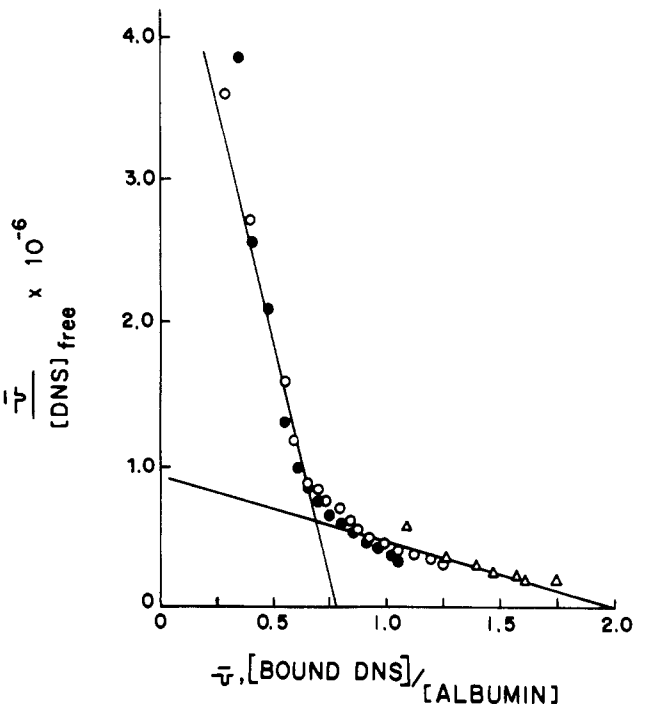

FIGURE 3: Scatchard analysis of DNS binding to HSA. Increasing amounts of 1.09 μM HSA were added to 2-mL solutions of 4 (○), 5 (●), and 10 μM (△) DNS. The $[DNS_{free}]/[DNS_{bound}]$ ratio was calculated from the fluorescence intensities at 425 nm. The value for the fluorescence intensity of bound DNS was that observed after correction for dilution, when additional albumin caused no further changes in intensity, $\bar{\nu}$ is the $[DNS_{bound}]/[albumin]$ ratio. Experimental conditions are described in the legend to Figure 1.

The solvent-dependent fluorescence properties of DNS are illustrated in Figure 2. The plots of the DNS wavelength maxima vs. the S scale (Brownstein, 1960), the Ω scale (Berson et al., 1962), the Z scale (Kosower, 1958), and the ϵ_T scale (Dimroth et al., 1963) were comparable. From the observed wavelength maximum of each complex of solvent and fluorophore, linear regression analysis of these data allowed an estimation of the polarity of the DNS binding regions on BSA and HSA. The S values were −0.165 and −0.183, the Ω values were 0.612 and 0.582, the Z values were 68.9 and 67.4 cal mol^{-1}, and the ϵ_T values were 40.2 and 39.0 cal mol^{-1} for BSA and HSA, respectively. The correlation coefficients of the data with the S, Ω, Z, and ϵ_T scales were 0.92, 0.96, 0.93, and 0.91, respectively.

The relative intensity at 425 nm was used to measure directly the concentration of bound DNS, since the probe in solution was virtually nonfluorescent at this wavelength. Scatchard analysis (Figure 3) indicated that, on HSA, there was one major binding site for DNS ($k_a \simeq 5 \times 10^6$) and one minor site ($k_a \simeq 3 \times 10^5$). The same values were obtained for BSA (data not shown). At low ratios of DNS to albumin, therefore, the site of lower affinity contained less than 10% of the total bound ligand. An isoemittive point similar to that in Figure 1 was found over the entire titration range of DNS, indicating that the probe in the low-affinity site had fluorescence behavior similar to that bound at the high-affinity binding site.

When increasing concentrations of homologous long chain saturated fatty acids were added to the fluorophore–albumin complex, DNS fluorescence intensity was decreased markedly by some, but not all, of the fatty acids (Figure 4). For example, the fluorescence at 425 nm decreased markedly when dodecanoate acid was added, whereas octadecanoate produced little change in the relative fluorescence intensity. In addition

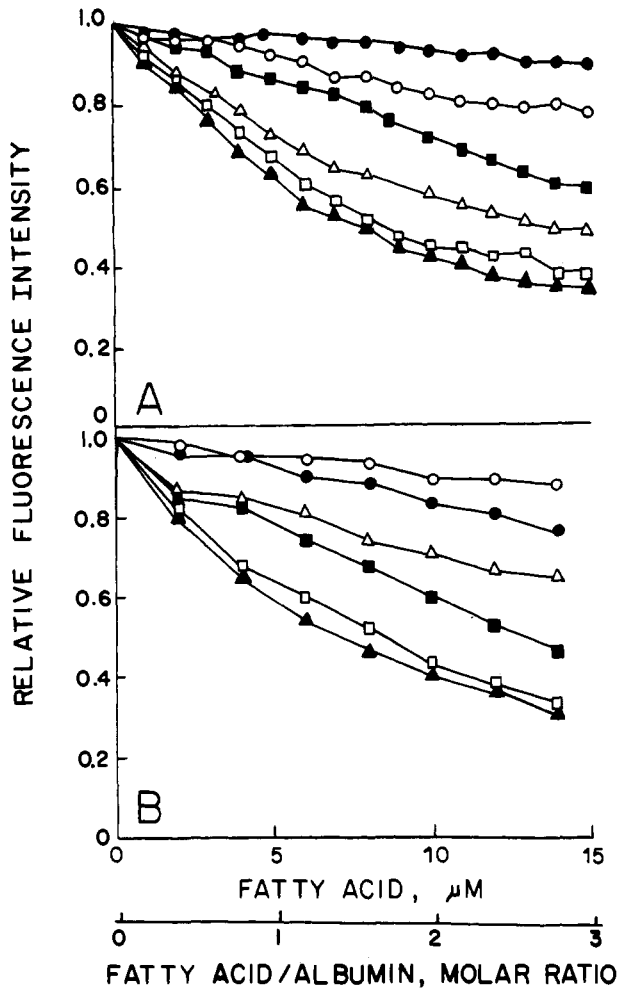

FIGURE 4: Effect of fatty acid and chain length on displacement of DNS from human and bovine serum albumin. Aliquots of 0.4 mM buffered fatty acid solutions, previously heated to solubilize the fatty acids, were added sequentially to 2-mL solutions of either 5 μM HSA (A) or BSA (B) containing 5 μM DNS. Fluorescence intensities at 425 nm were normalized to the fluorescence recorded prior to addition of fatty acid. Other experimental conditions are given in the legend to Figure 1: octadecanoate (●); hexadecanoate (○); tetradecanoate (■); dodecanoate (□); decanoate (▲); octanoate (△).

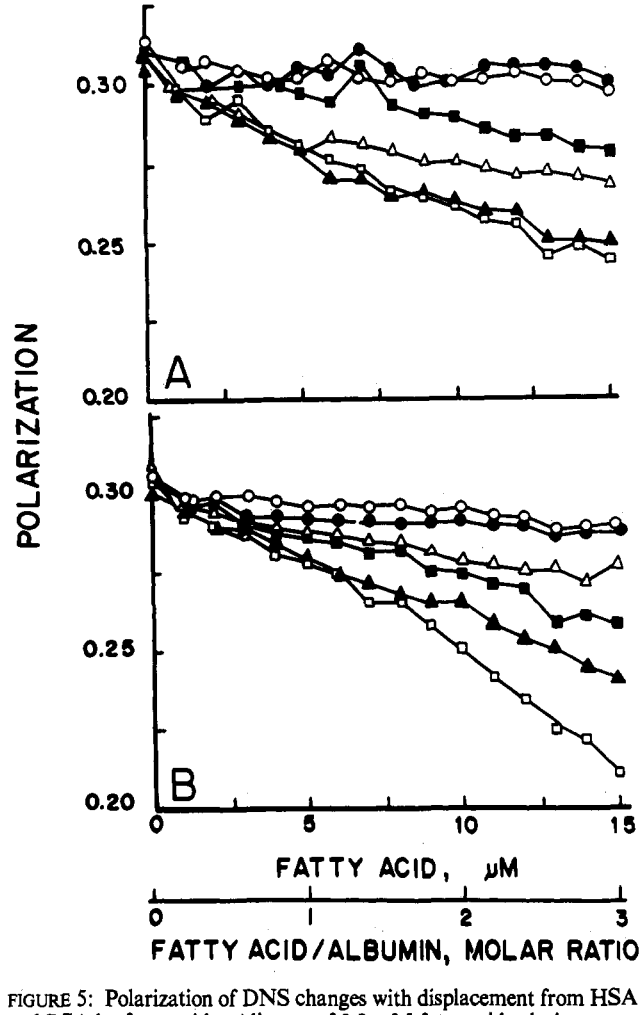

FIGURE 5: Polarization of DNS changes with displacement from HSA and BSA by fatty acids. Aliquots of 0.2 mM fatty acid solutions were added serially to 2 mL of 5 μM DNS and either 5 μM HSA (A) or 5 μM BSA (B) at 24 °C. After 5 min, polarization measurements were obtained in triplicate as described under Materials and Methods: octadecanoate (●); hexadecanoate (○); tetradecanoate (■); dodecanoate (□); decanoate (▲); octanoate (△).

to the observed decrease in relative intensity caused by increasing amounts of these fatty acids, the fluorescence wavelength maximum of DNS appeared at longer wavelengths and approached that of the probe in aqueous solution. Isoemissive points were observed with both HSA and BSA when fatty acids shifted the spectrum of the probe.

On a molar basis, the ability of fatty acids to displace DNS from HSA increased in the order decanoate > dodecanoate > octanoate > tetradecanoate > hexadecanoate > octadecanoate. For BSA, the order was decanoate > dodecanoate > tetradecanoate > octanoate > hexadecanoate > octadecanoate. Plots of these data, as 1/[displaced DNS] vs. 1/[FFA] (FFA represents free fatty acids), were linear. For all fatty acids and *N*-acetyl-L-tryptophan, each isotherm extrapolated to a common point of intersection corresponding to complete displacement of DNS, i.e., bound [DNS] = 0, thereby providing good evidence that, although they differed in affinity, all these ligands acted equivalently.

In addition to the blue shift and large increase in overall fluorescence intensity, the binding of DNS to either HSA or BSA caused large changes in fluorescence depolarization values. For aqueous DNS, the polarization value was virtually zero, 0.016 at 24 °C. The values of DNS noncovalently bound to HSA and BSA ranged between 0.300 and 0.315, comparable to that observed for DNS covalently attached to albumin (Weber & Young, 1964). Fatty acid induced changes in depolarization gave additional evidence that the DNS fluorescence changes accompaning fatty acid binding were due to displacement of DNS to the aqueous phase. As a function of fatty acid chain length and concentration, the relative ability to increase DNS depolarization increased in the order dodecanoate ≃ decanoate > octanoate > tetradecanoate > hexadecanoate ≃ octadecanoate for HSA. For BSA, the order was dodecanoate > decanoate > tetradecanoate > octanoate > hexadecanoate ≃ octadecanoate (Figure 5). Separate experiments established that *cis*-octadec-9-enoic acid (oleic acid) and *cis,cis*-octadec-9,12-dienoic acid (linoleic acid) were comparable to octadecanoic acid (steric acid) and essentially did not displace DNS from HSA. For *cis*-octadec-9-enoic acid, only 9% of the bound DNS was displaced; for *cis,cis*-octadec-9,12-dienoic acid, the value was 15%.

Fatty acids longer than 14 carbons have anomalous apparent p*K*s and self-associate in aqueous solutions (Smith & Tanford, 1973). Equilibrium constants for dimerization, trimerization, etc. are not available, since there are no satisfactory quantitative models for the self-association of fatty acids in aqueous solution (Simpson et al., 1974). The relative binding affinities of long chain and medium chain fatty acids are qualitative comparisons based on the experimentally determined distributions of fatty acids between individual binding sites on

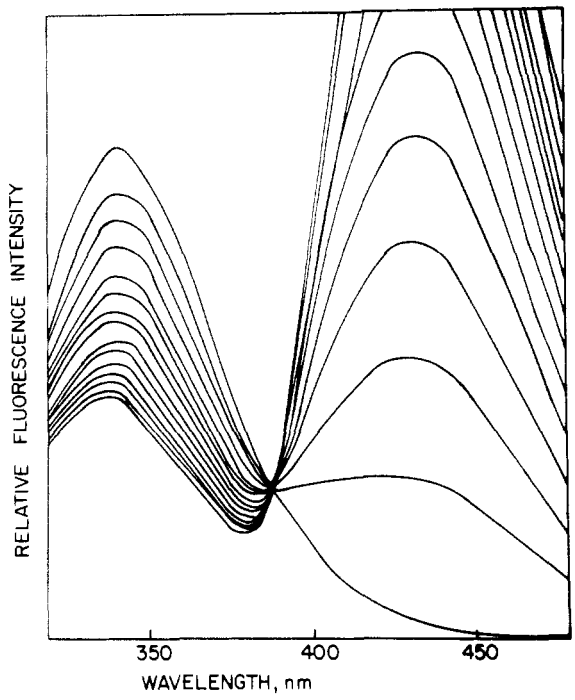

FIGURE 6: Energy transfer between HSA tryptophan and DNS. The upper trace at 340 nm was that of 5 μM HSA. The slight blue shift in the λ_{max} of protein fluorescence was due to tyrosine. Fifteen successive increments of 0.5 μM DNS were added as described under Materials and Methods. Excitation wavelength was 280 nm.

albumin and, in some cases, the multiple self-associated forms in water.

Resonance energy transfer from the tryptophan residue of HSA to bound DNS is demonstrated in Figure 6. With each addition of DNS, the tryptophan fluorescence decreased, with a corresponding increase in the fluorescence in the longer wavelength region of the spectrum. An isoemissive point was observed at 387 nm. A double-reciprocal plot of 1/(observed transfer efficiency) vs. 1/[DNS] gave a straight line (correlation coefficient = 0.99). The intercept corresponded to T = 0.69 at infinite DNS concentration (data not shown).

Discussion

The binding of DNS to albumin is demonstrated by several fluorescence parameters. Addition of albumin to solutions of DNS increases the overall fluorescence intensity of the DNS fluorescence maxima to shorter wavelengths and changes fluorescence polarization values. The isoemissive points indicate that only two forms of the probe are important. The regression of the spectrum of the DNS–albumin complex to the spectrum of DNS in the aqueous solution by the addition of fatty acid indicates that the titration produces unbound DNS, a conclusion substantiated by the concomitant increase in fluorescence depolarization.

As expected by analogy to the averaging of fluorescence anisotropy (Weber, 1953), fluorescence depolarization is less sensitive to displacement than is fluorescence intensity. In effect, polarization values are weighted toward that of the albumin-bound probe because of the nearly 4-fold difference in the relative fluorescence intensity of the probe in the two environments. Moreover, with the two methods, the relative abilities of fatty acid to displace DNS are the same. In addition to a convenient assay for DNS displacement from albumin by organic anions, these changes establish that the observed process is indeed a displacement rather than an allosterically induced perturbation of the microenvironment of the DNS binding site on albumin.

The uniqueness of the medium chain binding site is established by Scatchard analysis. Under the experimental conditions used, most of the protein-bound DNS is associated with the single high-affinity site ($k_a \simeq 5 \times 10^6$). DNS binding is different from that observed for the related molecule, ANS, which appears to have approximately six important binding sites (Santos & Spector, 1974). The linearity of double-reciprocal plots indicates that all of the fatty acids compete with dNS at its binding site, even though their ability to displace DNS decreases with increasing chain length and decreasing binding affinity. For both HSA and BSA, the most effective competitive fatty acids are decanoic and dodecanoic acid. The low affinities of the longer chain fatty acids with chain lengths greater than 14 carbons for this DNS site indicate that it is probably not an important physiological mechanism for the intravascular transport of these fatty acids. Albumin is unable to compete with lipoproteins for fatty acids when $\bar{\nu} > 4$ (Smith & Scow, 1979). The pattern of displacement observed with both proteins suggests the presence of a "hydrophobic-pocket" (Swaney & Klotz, 1970) at the DNS binding site with dimensions such that fatty acids with chain lengths greater than 12 carbons (1.8 nm) are effectively excluded. For HSA, Koh & Means (1979) found that dissociation constants for the series of fatty acids from butanoate through decanoate decreased from 3.2×10^{-4} to 1×10^{-7} M at a binding site with a capacity for nine methylene groups. Lee & McMenamy (1978) report that binding of long chain fatty acids to HSA increases the binding affinity at the medium chain fatty acid binding site. Presumably, this effect is mediated by a refolding process or an electrostatic change. This alteration produced by long chain fatty acids was not detected by DNS; however, such an effect cannot be excluded. In addition, their finding that *N*-acetyl-L-tryptophan binds at the medium chain fatty acid binding site is supported by our finding that *N*-acetyl-L-tryptophan displaces DNS from HSA.

Shifts of emission spectra to shorter wavelengths and increases in quantum yield are generally accepted as spectroscopic indicators of movement of probe molecules from a polar to a less polar environment. Environmental "polarity" is determined by the sum of all intermolecular forces and, as such, is not fully defined by a single parameter. For this reason, the previously derived polarity scales originate from theoretical treatments of the overall effect of solvents on processes such as reaction kinetics and the spectroscopic behavior of standard chromophores (Kosower, 1958; Brownstein, 1960; Dimroth et al., 1963; Berson et al., 1962). These four scales are highly corrected and have considerable practical importance. A comprehensive treatment of this subject is available by Reichardt (1978).

These polarity values determined for the DNS binding sites on HSA and BSA are consistent with a highly nonpolar environement. This site is considerably less polar than the hydrocarbon region of artificial lipid bilayers (Kao et al., 1978; Craig et al., 1981). The combination of very low polarity and electrostatic interactions (Oakes, 1973) at this site presumably would enable albumin to compete with plasma lipoproteins for medium chain fatty acids and for other small nonpolar negatively charged organic compounds.

Energy transfer through a Förster (1959) mechanism occurs from the tryptophan of HSA to DNS bound at the medium chain fatty acid site. It is possible to define a relative quantum yield, Q_R, for each addition, where

$$Q_R = I/I_0$$

where I and I_0 are the fluorescence intensities at a representative wavelength of the quenched and unquenched solu-

tions, respectively. Energy transfer efficiency (T) is then defined as

$$T = 1 - Q_R$$

Under conditions that would be expected to saturate the primary DNS site without significant binding of DNS to the secondary sites, the transfer efficiency extrapolated to a value of 0.69. A value of 20.7 ± 2 Å is obtained for the distance between the tryptophan of HSA and the high-affinity DNS binding site from

$$R = \left[R_0^6 \left(\frac{1 - T}{T} \right) \right]^{1/6}$$

Berde et al. (1979) report a distance of 24–25 Å between the tryptophan of HSA and the primary binding site of the naturally occuring long chain fatty acids *cis*-parinaric acid (*cis,trans,trans,cis*-9,11,13,15-octadecatetraenoic acid) and eleostearic acid (*all-cis*-9,11,13-octadecatrienoic acid).

Long chain fatty acids of dietary origin enter the circulation as chylomicron triglycerides and are subsequently transferred as fatty acids into peripheral tissues after lipoprotein lipase action on the lipoprotein substrate (Robinson, 1970; Scow et al., 1976). By contrast, medium chain fatty acids from the diet are transported from the intestine primarily by the portal vascular circulation directly to the liver (Kiyasu et al., 1952; Bergström, 1955). A highly specific high-affinity medium chain binding site on albumin may be the mechanism by which this selective transport is accomplished. It appears that the medium chain site does not accomodate the more abundant and more hydrophobic long chain fatty acids.

The presence of a nearly identical binding site on both human and bovine albumin for medium chain fatty acids and DNS indicates that the physiological roles are important enough to warrant evolutionary conservation. Studies of the more primitive serum albumins may eventually reveal whether the medium chain site evolved through modification of preexisting nonselective sites or whether it evolved independently by modification of unrelated structural regions.

Acknowledgments

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