Conformational changes in human serum albumin studied by fluorescence and absorption spectroscopy

Distance measurements as a function of pH and fatty acids

Bent HONORÉ* and Anders O. PEDERSEN

Institute of Medical Biochemistry, University of Aarhus, DK-8000 Aarhus C, Denmark

pH- and fatty acid-induced conformational changes in human serum albumin were investigated by fluorescence-energy transfer, determining the distance between Trp-214 and bound bilirubin at 25 °C. This distance changes significantly with the pH, being 2.52 ± 0.01 nm at pH 6, 2.31 ± 0.04 nm at pH 9, 2.13 ± 0.07 nm at pH 11.0 and 2.77 nm at pH 11.9. The influence of different fatty acids on the distance was also determined. At pH 7.4 medium-chain fatty acids seem to increase this distance, whereas long-chain fatty acids, at low concentrations, decrease the distance between the two chromophores. The contraction of the protein carrying long-chain saturated fatty acids is even more pronounced at pH 9.

INTRODUCTION

Human serum albumin is the most abundant protein circulating in blood, transporting a number of ligands (Peters, 1985). Bilirubin, fatty acids, L-tryptophan, some hormones and a large variety of chemically different drugs bind to albumin (Kragh-Hansen, 1981). Albumin contains 585 amino acid residues in a single chain with known sequence (Meloun *et al.*, 1975). A model of albumin consisting of six half-domains has been suggested (Brown & Shockley, 1982). So far, however, solid evidence for this model remains modest, because X-ray-crystallographic investigations have been unable to resolve molecular details. Structural information has thus been obtained indirectly by studying selected properties of the albumin molecule on interaction with the environment, the most thoroughly studied being pH-dependent phenomena (Foster, 1977).

Conformational changes may be studied by means of excitation-energy transfer between a fluorescent donor and an absorbing acceptor, since the efficiency of transfer is dependent on the distance between the two chromophores. Human serum albumin is especially suitable in this respect since it contains only one tryptophan residue (Trp-214), which accounts for all the fluorescence of albumin when excited at 295 nm, tyrosine fluorescence being negligible (Teale, 1960). Thus the sole tryptophan residue can serve as a structural reference point in energy transfer to suitable acceptor chromophores attached to the albumin molecule. Darnall and co-workers (Suzukida *et al.*, 1983; Hagag *et al.*, 1983) have determined distances between Trp-214, Cys-34 and Tyr-411 after labelling the two latter with an azomercurial and *p*-nitrophenyl anthranilate respectively. At neutral pH they found the distance between Cys-34 and Trp-214 to be 3.18 ± 0.08 nm, that between Trp-214 and Tyr-411 to be 2.52 ± 0.06 nm and that between Cys-34 and Tyr-411 to be 2.52 ± 0.21 nm. One of the distances, i.e. Cys-34 to Trp-214, was measured at different pH values between 2 and 9, and it was found that this distance decreased by 0.4–0.5 nm during the N (pH 6)-to-F (pH 4) transition

and decreased by 0.3–0.4 nm during the N-to-B (pH 9) transition.

In the present study the tightly bound bilirubin was chosen as the absorbing acceptor chromophore (Brodersen *et al.*, 1987). Since the bilirubin–human serum albumin complex undergoes several conformational transitions between pH 6 and 12 (Blauer *et al.*, 1972; Jacobsen & Færch, 1980; Honoré & Frandsen, 1986; Honoré, 1987), it was decided to study the distance between Trp-214 and bound bilirubin in this pH interval.

Binding of fatty acids to albumin as well as to the albumin–bilirubin complex also induces conformational changes (Spector, 1975; Brodersen, 1979). Influence on the distance between Trp-214 and bound bilirubin was studied with different saturated and unsaturated fatty acids.

EXPERIMENTAL

Materials

Human serum albumin. This was obtained from AB Kabi (Stockholm, Sweden). It was defatted with charcoal (Chen, 1967a) in solution at 0 °C, acidified with H₂SO₄ to pH 3 and then freeze-dried.

Bilirubin. This was obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). It was purified by the procedure of McDonagh & Assisi (1972), giving a preparation where 95% of the isomers is bilirubin IX- α (ZZ). A 1.30 mM alkaline solution was prepared as described previously (Honoré & Frandsen, 1986), and a calculated amount was immediately mixed with the buffered albumin at pH 9. All experiments with bilirubin were conducted in orange light to avoid photodecomposition.

Fatty acids. Hexadec-9-enoate (palmitoleate), octadec-9-enoate (oleate), octadeca-9,12-dienoate (linoleate), octadeca-9,12,15-trienoate (linolenate) and hexadeca-enoate (palmitate) were from Fluka A.G. (Buchs, Switzerland). Octadec-11-enoate (vaccenate) was from

* To whom correspondence should be addressed.

Sigma Chemical Co. and octadecanoate (stearate) was from Merck A.G. (Darmstadt, Germany). These fatty acids were dissolved in ethanol and added, at pH 9, up to a ratio of 4 mol/mol of albumin and a final ethanol concentration 5 ml/l. The pH was finally adjusted. A separate experiment showed that this ethanol concentration did not influence the measured distance. Tetradecanoate (myristate), dodecanoate (laurate), decanoate (caprate) and hexanoate (caproate) were from Fluka. Octanoate (caprylate) was from Merck. These acids were dissolved in 60 mM-NaOH, diluted with water and added to the buffered albumin at neutral or alkaline pH up to a ratio of 5 mol/mol of albumin. Stock solutions containing unsaturated fatty acids were flushed with N₂ to avoid oxidation.

Methods

Measurement of pH. At pH values between 6 and 10 the measurements were done with a combination electrode type GK2320C from Radiometer. At higher pH values this electrode possesses sodium error and consequently a glass electrode type G2040B from Radiometer was used at and above pH 11 as described previously (Honoré & Frandsen, 1986).

Preparation of solutions. Albumin solutions were made by weighing, the M_r being taken as 66000. Albumin was dissolved in 0.15 M-NaCl in 10 mM buffer. Different buffers were used depending on the final pH, namely sodium phosphate buffer (Merck) at pH 7.4, Tris/HCl buffer (Sigma) at pH 6–10 and glycine/NaOH buffer (Merck) at pH 11–12.1. In each case a stock solution of albumin was prepared with a pH value of 9. This solution was divided into two portions, with alkaline bilirubin added to one albumin solution and an equal amount of water to the other. Each of the two albumin solutions was finally diluted 15-fold with 0.15 M-NaCl in 10 mM buffer. Two albumin solutions, 4 μ M, were thereby obtained, one without bilirubin and one with 2 μ M-bilirubin, in some cases 1 μ M-bilirubin. The pH was finally adjusted to the desired value, and experiments were performed immediately afterwards to ensure that spectral measurements were carried out at the pH stated.

The temperature in all experiments was 25 °C.

Light-absorption spectra. Light-absorption was measured in 4 cm cuvettes on an ACTA mark VI spectrophotometer from Beckman Instruments (Fullerton, CA, U.S.A.).

Fluorescence spectra. Corrected fluorescence spectra were recorded on an RF-540 spectrofluorophotometer from Shimadzu Corporation (Tokyo, Japan) with rhodamine B as quantum counter. Measurements were done in 10 mm $\times$ 10 mm or 5 mm $\times$ 5 mm cells. Inner-filter effects were corrected for by multiplying the observed fluorescence intensities by $10^{(A_{exc.} + A_{em.})/2}$, where $A_{exc.}$ and $A_{em.}$ are the absorbances at the excitation and emission wavelengths, measured at the cell length used. This correction is considered to be accurate within 1% when the absorbance is kept below 0.2 (Birdsall *et al.*, 1983). The excitation wavelength was 295 nm in order to minimize the contribution of tyrosine residues to the albumin fluorescence, which then solely originates from Trp-214 (Teale, 1960). The Raman emission (Stoke's

lines) around 330 nm was eliminated by subtracting the fluorescence of a blank. The Rayleigh scattering at 295 nm was eliminated by linear interpolation between fluorescence intensities at 285 nm and 310 nm.

The quantum yield of emission from albumin, Q , was measured by the comparative method (Chen *et al.*, 1969):

$$Q = Q_{st.} \cdot \frac{F}{F_{st.}} \cdot \frac{A_{st.}}{A} \quad (1)$$

where F is the area under the fluorescence spectrum of albumin and A the absorbance of albumin at the excitation wavelength. $Q_{st.}$, $F_{st.}$ and $A_{st.}$ are the corresponding parameters for the standard, excited at the same wavelength, in this case quinine sulphate (Fluka) with the quantum yield 0.70 (Scott *et al.*, 1970). It was assumed that ionization of tyrosine residues above pH 10 did not influence the absorption of the tryptophan residue at 295 nm. This assumption is justified by the observation of an emission maximum at the same wavelength, 340 nm, at all pH values (Teale, 1960; Burstein *et al.*, 1973).

Distance calculations. The quantum yield of fluorescence from the tryptophan chromophore also depends upon a multitude of environmental factors, such as collisional inactivation, photochemical reactions and radiationless transfer of excitation energy from the tryptophan chromophore to a suitable acceptor chromophore, e.g. bilirubin. According to the Förster (1948) theory, transfer of singlet-excitation energy via the dipole resonance mechanism depends on the spectral overlap integral between donor and acceptor chromophores and, most importantly, the distances between the two chromophores:

$$R = R_0[(1 - E)/E]^{1/6} \text{ nm} \quad (2)$$

The quenching parameter, E , indicating quenching efficiency of the liganded bilirubin on tryptophan emission, is obtained directly from experimental data of fluorescence intensity:

$$E = 1 - \frac{F_B}{F_0} \quad (3a)$$

where F_B and F_0 are the integrated intensities of tryptophan emission in the presence and in the absence of bilirubin respectively. A corrected value of quenching efficiency, E_c , corresponding to equal concentrations of emitter and quencher, was approximated by:

$$E_c = \frac{E}{f} \quad (3b)$$

where f is the fraction of albumin molecules containing bilirubin (Fairclough & Cantor, 1978). The parameter f depends upon the affinity of bilirubin for albumin. The affinity is constant, 10^7 – 10^8 M⁻¹, at pH values between 6 and 9 (Jacobsen 1969; Jacobsen & Brodersen, 1976), as measured by the peroxidase technique (Brodersen, 1974), which is applicable up to pH 9.3. At higher pH values the affinity was determined from fluorescence measurements of free and bound bilirubin to be 10^7 – 10^8 M⁻¹ at pH 10.0, 3×10^6 M⁻¹ at pH 11.0, 8×10^5 M⁻¹ at pH 11.3 and 3×10^5 M⁻¹ at pH 11.9. From these affinities and the known total concentrations of bilirubin and albumin, f was calculated at each pH value.

The distance parameter, R_0 , can be elaborated from

spectral data of donor and acceptor as follows (Stryer, 1978):

$$R_0 = 970(J \cdot K^2 \cdot Q_0 \cdot n^{-4})^{\frac{1}{6}} \text{ nm} \quad (4)$$

J , the spectral overlap integral, expresses the probability of resonance between the oscillating dipoles in the tryptophan and bilirubin chromophores, and is calculated from:

$$J = \frac{\int_0^\infty F(w) \cdot \epsilon(w) \cdot w^4 \cdot dw}{\int_0^\infty F(w) \cdot dw} \text{ cm}^3 \cdot \text{M}^{-1} \quad (5)$$

where $F(w)$ is the intensity of emission from the tryptophan group, $\epsilon(w)$ is the absorption coefficient ($\text{M}^{-1} \cdot \text{cm}^{-1}$) of the bound bilirubin, and w is the wavelength (cm). K^2 is an orientation factor and was given the value $\frac{2}{3}$ under the assumption of random mutual orientation of the tryptophan chromophore and the bilirubin chromophore. This assumption is justified by a high degree of mobility of the tryptophan group (Munro *et al.*, 1979; Lakowicz & Weber, 1980). Q_0 is the quantum yield of emission from the tryptophan group in the absence of bilirubin. The refractive index of the non-absorptive space between the emitter and quencher is n .

RESULTS

Albumin fluorescence at various pH values

Fluorescence spectra of albumin ($4 \mu\text{M}$) with and without bilirubin ($2 \mu\text{M}$) at various pH values are shown in Fig. 1. The emission maximum is located at 340 nm at all pH values. The fluorescence quantum yield, Q , of tryptophan at neutral pH is 0.15–0.18 and decreases as the pH is raised above 10 (Table 1). This is probably due to excitation transfer from tryptophan to ionized tyrosine residues (Chen, 1967*b*), where it is dissipated as heat after collisional inactivation with solvent water.

Quenching of albumin fluorescence by bilirubin

Addition of bilirubin to albumin in the molar ratio

0.5:1 strongly quenches (by 30–35%) the tryptophan fluorescence at neutral and slightly alkaline pH values. At these pH values, bilirubin is tightly bound to albumin with the first stoichiometric binding constant 10^7 – 10^8 M^{-1} (Jacobsen, 1969; Jacobsen & Brodersen, 1976). At pH 11.9 the quenching effect of bilirubin is only weak (5–10%) (Fig. 1). This is explained only in part by a decreasing affinity of bilirubin for albumin with the increasing pH (see the Experimental section). The change in quenching efficiency by bilirubin at various pH values must therefore be supplemented with a different explanation. Two possible mechanisms should be considered. First, bilirubin binding might induce conformational changes in the protein resulting in different rates of collisional inactivation of the excited tryptophan chromophore at different pH values. This seems unlikely for the following reasons. Modification of the lone tryptophan residue does not change the affinity for bilirubin (Jacobsen, 1972), and addition of bilirubin does not change the wavelength of the emission maximum of albumin at any of the pH values examined. The position of the emission maximum, 340 nm, suggests that Trp-214 is located near the surface of the molecule in limited contact with water (Burstein *et al.*, 1973). A conformational change in the neighbourhood of the tryptophan residue is likely to result in a shift of this maximum, as observed upon addition of fatty acids in the presence of Ca^{2+} (Aguanno & Ladenson, 1982) or following glycosylation of Lys-525 (Shaklai *et al.*, 1984). However, the changes in bilirubin quenching with different pH values can be explained by variations in excitation transfer from tryptophan to bilirubin. This is possible, since the absorption spectra of bound bilirubin at various pH values overlap with the tryptophan fluorescence spectra (Fig. 1) in the pH range studied.

Distance between Trp-214 and bound bilirubin

Distance variation with pH. From the spectral data, the overlap integrals were calculated by using eqn. (5). Assuming random orientation of the chromophores, i.e. $K^2 = \frac{2}{3}$, and giving the refractive index of the non-

Fig. 1. Fluorescence and light-absorption spectra of bilirubin-albumin at different pH values

Continuous curves show quantum-corrected fluorescence spectra of albumin ($4 \mu\text{M}$) (upper spectrum at each pH) and with bilirubin ($2 \mu\text{M}$) added (lower spectrum). Excitation was at 295 nm. Broken curves show the absorption of albumin-bound bilirubin. Solutions were in 150 mM-NaCl in 10 mM buffer (see the Experimental section). The temperature was 25 °C.

Table 1. Distance between Trp-214 in human serum albumin and bound bilirubin at various pH values

Equations used for calculations are given in the Experimental section. [B]/[A] represents the ratio of micromolar concentrations of bilirubin [B] and albumin [A].

pH	[B]/[A]	Q	E_c	$10^{14} \times J$ ($M^{-1} \cdot cm^3$)	R_0 (nm)	R (nm)	
						Mean $\pm$ S.D.	
6.0	1/4	0.14	0.61	1.94	2.72	2.52	2.52 $\pm$ 0.01
	2/4	0.16	0.64	1.91	2.76	2.51	
7.0	1/4	0.17	0.60	1.77	2.74	2.56	2.56
7.4	1/4	0.16	0.68	1.94	2.77	2.44	2.44 $\pm$ 0.01
	1/4	0.16	0.67	1.97	2.78	2.46	
	2/4	0.17	0.69	1.95	2.80	2.44	
	2/4	0.15	0.64	1.69	2.67	2.43	
	2/4	0.18	0.70	1.92	2.81	2.44	
	2/4*	0.18	0.71	2.02	2.83	2.44	
9.0	1/4	0.16	0.71	1.84	2.75	2.37	2.31 $\pm$ 0.04
	1/4	0.17	0.78	2.03	2.82	2.29	
	2/4	0.18	0.76	1.92	2.81	2.31	
	2/4	0.18	0.77	1.82	2.78	2.28	
10.0	1/4	0.14	0.68	1.83	2.67	2.35	2.32 $\pm$ 0.05
	2/4	0.15	0.73	1.74	2.69	2.28	
11.0	2/4	0.11	0.77	1.65	2.54	2.08	2.13 $\pm$ 0.07
	2/4	0.12	0.73	1.72	2.58	2.18	
11.3	1/4	0.11	0.61	2.56	2.73	2.53	2.58 $\pm$ 0.06
	2/4	0.11	0.58	2.71	2.75	2.62	
11.9	2/4	0.072	0.36	2.44	2.52	2.77	2.77

* Experiment obtained with an ethanol concentration 5 ml/l.

Fig. 2. Effect of different fatty acids on the distance between Trp-214 in human serum albumin and bilirubin

The numbers in parentheses indicate the number of carbon atoms in the fatty acids added with the molar ratio given in the abscissa. The bars are 1 S.D. Conditions were as indicated in Fig. 1 legend.

absorptive protein matrix between the chromophores the value 1.4 (Fairclough & Cantor, 1978), the distance between the chromophores was calculated at different pH values, as shown in Table 1 and as described in the Experimental section. For control, the distance calculations were occasionally carried out at various bilirubin/albumin ratios, but giving similar values for the distance, providing evidence that the procedure for calculating E_c is reasonable (Table 1). The standard deviation of the calculations is 0.07 nm or better. The distance at pH 6 is 2.52 ± 0.01 nm, significantly decreasing as pH increases, to 2.31 ± 0.04 nm at pH 9 and further decreasing to 2.13 ± 0.07 nm at pH 11.0. The neutral-to-basic transition (pH 6 to pH 9) thus involves a contraction of the distance by about 0.2 nm. On the other hand the distance seems to increase by 0.6–0.7 nm when the pH is increased from 11.0 to 11.9.

Change in distance with various concentrations of different fatty acids bound at neutral pH. The distance was determined to be 2.44 ± 0.01 nm without fatty acids bound. Fig. 2 shows how this distance changes upon addition of different fatty acids to albumin. The medium-chain fatty acids generally increase the distance. The long-chain acids (C_{12} to C_{18}), on the other hand, decrease the distance when present in low concentrations. Further conclusions concerning the effect of chain length and saturation of the fatty acid cannot be made from these results. This is seen from the fact that the effect on the distance of 2 mol of fatty acid/mol generally is different from the effect of 4 mol of fatty acid/mol (Fig. 2). For example, addition of 2 mol of octadecanoate

Table 2. Distance between Trp-214 in human serum albumin and bound bilirubin with 2 mol of saturated fatty acid/mol of albumin at different pH values

The change in R is the distance with 2 mol of fatty acids/mol of albumin minus the distance without fatty acids.

Fatty acid	R (nm)			Change in R (nm)		
	pH 6	pH 7.4	pH 9	pH 6	pH 7.4	pH 9
None*	2.52±0.01	2.44±0.01	2.31±0.04			
Hexanoate	2.37	2.47	2.19	-0.15	0.03	-0.13
Octanoate	2.37	2.49	2.10	-0.14	0.05	-0.22
Decanoate	2.40	2.44	1.96	-0.12	0.00	-0.36
Dodecanoate	2.36	2.41	1.80	-0.15	-0.04	-0.51
Tetradecanoate	2.43	2.29±0.03	1.96	-0.09	-0.15	-0.36
Hexadecanoate	2.52	2.35±0.01	2.08	0.01	-0.09	-0.24
Octadecanoate	2.55	2.25±0.05	2.19	0.03	-0.19	-0.13

* Values taken from Table 1.

(C₁₈)/mol decreases the distance more than does 2 mol of tetradecanoate (C₁₄)/mol, whereas addition of 4 mol of octadecanoate/mol decreases the distance less than does 4 mol of tetradecanoate/mol.

Change in distance with various fatty acids at different pH values. Table 2 shows the effect of 2 mol of different saturated fatty acids/mol on the distance at pH 6, 7.4 and 9. The effect on the distance clearly depends on pH, being more pronounced at alkaline pH with fatty acids containing more than eight carbon atoms. At pH 9 they decrease the distance up to 0.5 nm, whereas the decreasing effect is below 0.2 nm at pH 6.

DISCUSSION

The fluorescence-energy transfer experiments are subject to several kinds of systematic errors. Absolute measurement of quantum yield via actinometry is a complicated matter. It is therefore common to measure the quantum yield relative to a known standard. Unfortunately the literature does not always agree on this type of information. The quantum yield of tryptophan has been reported between 0.13 (Chen *et al.*, 1969) and 0.20 (Teale & Weber, 1957) at an unspecified temperature. Since the quantum yield of tryptophan is temperature-sensitive, changing about 2% per degree (Gally & Edelman, 1964), this is a less suitable standard. In the present study quinine sulphate was chosen as a standard. The quantum yield has been determined by Scott *et al.* (1970) to be 0.70 at 25 °C by two different methods. A lower value, 0.55, was reported by Melhuish (1955). If the latter value is correct compared with the former, the distance calculations will be overestimated by about 0.1 nm, as seen from eqn. (1) and eqn. (4).

The orientation factor K^2 in eqn. (4) may assume values between 0 and 4. At first glance this might seem an obstacle in calculating the distances. It is often possible, however, to assume that the mutual orientation of the donor and acceptor chromophore is random, in which case $K^2 = \frac{2}{3}$ (Stryer, 1978). Haas *et al.* (1978) have shown that this is indeed a good estimate when one or both of the chromophores show mixed polarizations, thus characterized by two or more transition dipoles. This applies for both the tryptophan chromophore (Valeur & Weber, 1977) and the bilirubin chromophore (Blauer

et al., 1972). More importantly, Munro *et al.* (1979) and Lakowicz & Weber (1980) have shown that the tryptophan residue in human serum albumin rotates rapidly relative to the complete protein molecule. Thus the random orientation of the donor chromophore relative to the liganded bilirubin chromophore is reasonably substantiated.

Regarding other sources of error, inspection of eqn. (2) reveals that precision in determination of R is maximal for $R = R_0$, corresponding to 50% quenching. Fortunately, the investigated pair of donor and acceptor possesses spectral properties in agreement with this criterion, and we end up with standard deviations below 0.07 nm in all pH experiments and in most experiments with added fatty acids. Darnall and co-workers (Suzukida *et al.*, 1983; Hagag *et al.*, 1983) report standard deviations between 0.06 and 0.21 nm for the distances between Cys-34, Trp-214 and Tyr-411. The present system is thus excellent for studying the effect of other ligands on the conformation of albumin by measuring changes in the distance. In fact, the observed changes in the distance measurements may be more reliable than the absolute values, since the errors will be minimized by subtraction (Suzukida *et al.*, 1983). In the following, attention is focused on changes in the distance.

Distance between Trp-214 and bound bilirubin at various pH values

There is evidence suggesting that bilirubin is bound in the N -terminal half between the second and third half-domains of albumin, probably involving Lys-240 (Jacobsen, 1978) and residues 195–197 (Reed & MacKay, 1985). Around neutral pH the distance between bilirubin and Trp-214 is calculated to be 2.44 nm (Table 1). In the literature, the values 2.8 nm (Chen, 1973), 2.7 nm (Berde *et al.*, 1979) at neutral pH and 2.2 nm (Brodersen *et al.*, 1987) at slightly alkaline pH have been reported. Table 1 shows that a small but significant decrease in the distance occurs during the N (pH 6)-to-B (pH 9) transition of albumin (Leonard *et al.*, 1963), i.e. a 0.2 nm decrease from 2.5 to 2.3 nm. Suzukida *et al.* (1983) studied the distance between Trp-214 and an azo compound bound to Cys-34 at different pH values. They found that this distance decreased from 3.4–3.5 nm at pH 6 to 3.1–3.2 nm at pH 9. In connection with the present measurements, this indicates that parts of the

N-terminal of the albumin molecule contract during the neutral-to-basic transition. With a further increase of pH from 10.0 to 11.0 the distance decreases by about 0.2 nm to a minimum of 2.13 nm.

As the pH is raised from 11.0 to 11.3 the distance increases sharply by 0.4–0.5 nm. From pH 11.3 to 11.9 in the highly cooperative first alkaline transition (Honoré & Frandsen, 1986) the distance increases by 0.2 nm. These observations are in agreement with the previous assumption that an unfolding of this section of the albumin molecule occurs at this high pH (Honoré & Frandsen, 1986).

Distance between Trp-214 and bound bilirubin at various concentrations of fatty acids bound

Up to four molecules of fatty acids can generally be bound per molecule without displacing bilirubin (Brodersen, 1979). As shown in Fig. 2, the fatty acids are capable of inducing conformational changes in the protein, altering the distance between the lone tryptophan residue and bilirubin. This effect is largest at alkaline pH. It is not possible, however to extract firm conclusions about conformational changes as a function of chain length and saturation of the fatty acids added.

We are indebted to Professor Rolf Brodersen for stimulating discussions and criticism of the manuscript. The technical assistance of Nina Jørgensen is highly appreciated. Thanks are due to Frede Nielsen, who kindly made the drawings. This work was supported by the Danish Medical Research Council and Aarhus Universitets Forskningsfond (B. H.). An HP-85 desk computer and a Shimadzu RF-540 spectrofluorophotometer were obtained through Grants 12-2365 and 12-7126 from the Danish Medical Research Council to R. Brodersen.

REFERENCES

- Aguanno, J. J. & Ladenson, J. H. (1982) *J. Biol. Chem.* **257**, 8745–8748
- Berde, C. B., Hudson, B. S., Simoni, R. D. & Sklar, L. A. (1979) *J. Biol. Chem.* **254**, 391–400
- Birdsall, B., King, R. W., Wheeler, M. R., Lewis, C. A., Jr., Goode, S. R., Dunlap, R. B. & Roberts, G. C. K. (1983) *Anal. Biochem.* **132**, 353–361
- Blauer, G., Harmatz, D. & Snir, J. (1972) *Biochim. Biophys. Acta* **278**, 68–88
- Brodersen, R. (1974) *J. Clin. Invest.* **54**, 1353–1364
- Brodersen, R. (1979) *CRC Crit. Rev. Clin. Lab. Sci.* **11**, 305–399
- Brodersen, R., Knudsen, A. & Pedersen, A. O. (1987) *Arch. Biochem. Biophys.* **252**, 561–569
- Brown, J. R. & Shockley, P. (1982) in *Lipid-Protein Interactions* (Jost, P. C. & Griffith, O. H., eds.), vol. 1, pp. 25–68, John Wiley and Sons, New York
- Burstein, E. A., Vedenkina, N. S. & Ivkova, M. N. (1973) *Photochem. Photobiol.* **18**, 263–279
- Chen, R. F. (1967a) *J. Biol. Chem.* **242**, 173–181
- Chen, R. F. (1967b) in *Fluorescence: Theory, Instrumentation and Practice* (Guilbault, G. G., ed.), pp. 443–509, Marcel Dekker, New York
- Chen, R. F. (1973) in *Fluorescence Techniques in Cell Biology* (Thaer, A. A. & Sernetz, M., eds.), pp. 273–282, Springer-Verlag, New York
- Chen, R. F., Edelhoch, H. & Steiner, R. F. (1969) in *Physical Principles and Techniques of Protein Chemistry* (Leach, S. J., ed.), part A, pp. 171–244, Academic Press, New York
- Fairclough, R. H. & Cantor, C. R. (1978) *Methods Enzymol.* **48**, 347–379
- Förster, T. (1948) *Ann. Phys. (Leipzig)* **2**, 55–75
- Foster, J. F. (1977) in *Albumin Structure, Function and Uses* (Rosenoer, V. M., Oratz, M. & Rothschild, M. A., eds.), pp. 53–84, Pergamon Press, Elmsford
- Gally, J. A. & Edelman, G. M. (1964) *Biopolym. Symp.* **1**, 367–381
- Haas, E., Katchalski-Katzir, E. & Steinberg, I. Z. (1978) *Biochemistry* **17**, 5064–5070
- Hagag, N., Birnbaum, E. R. & Darnall, D. W. (1983) *Biochemistry* **22**, 2420–2427
- Honoré, B. (1987) *J. Biol. Chem.* **262**, 14939–14944
- Honoré, B. & Frandsen, P. C. (1986) *Biochem. J.* **236**, 365–369
- Jacobsen, C. (1972) *Eur. J. Biochem.* **27**, 513–519
- Jacobsen, C. (1978) *Biochem. J.* **171**, 453–459
- Jacobsen, J. (1969) *FEBS Lett.* **5**, 112–114
- Jacobsen, J. & Brodersen, B. (1976) *Birth Defects Orig. Art. Ser.* **12**, 175–178
- Jacobsen, J. & Færch, T. (1980) *Biochim. Biophys. Acta* **623**, 199–207
- Kragh-Hansen, U. (1981) *Pharmacol. Rev.* **33**, 17–53
- Lakowicz, J. R. & Weber, G. (1980) *Biophys. J.* **30**, 591–601
- Leonard, W. J., Jr., Vijai, K. K. & Foster, J. F. (1963) *J. Biol. Chem.* **238**, 1984–1988
- McDonagh, A. F. & Assisi, F. (1972) *Biochem. J.* **129**, 797–800
- Melhuish, W. H. (1955) *N.Z. J. Sci. Technol.* **37B**, 142–149
- Meloun, B., Morávek, L. & Kostka, V. (1975) *FEBS Lett.* **58**, 134–137
- Munro, I., Pecht, I. & Stryer, L. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 56–60
- Peters, T., Jr. (1985) *Adv. Protein Chem.* **37**, 161–245
- Reed, R. G. & MacKay, C. M. (1985) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **44**, 1618
- Scott, T. G., Spencer, R. D., Leonard, N. J. & Weber, G. (1970) *J. Am. Chem. Soc.* **92**, 687–695
- Shaklai, N., Garlick, R. L. & Bunn, H. F. (1984) *J. Biol. Chem.* **259**, 3812–3817
- Spector, A. A. (1975) *J. Lipid Res.* **16**, 165–179
- Stryer, L. (1978) *Annu. Rev. Biochem.* **47**, 819–846
- Suzukida, M., Le, H. P., Shahid, F., McPherson, R. A., Birnbaum, E. R. & Darnall, D. W. (1983) *Biochemistry* **22**, 2415–2420
- Teale, F. W. J. (1960) *Biochem. J.* **76**, 381–388
- Teale, F. W. J. & Weber, G. (1957) *Biochem. J.* **65**, 476–482
- Valeur, B. & Weber, G. (1977) *Photochem. Photobiol.* **25**, 441–444