FLUORESCENCE STUDIES ON HUMAN SERUM ALBUMIN

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The fluorescence decay of the tryptophan emission of human serum albumin has been measured in various conditions. In all the cases studied a complex decay is observed, which can be described by a sum of two exponentials. At pH 5.5, the decay times are 3.3 ns and 7.8 ns ; the relative contributions of the exponentials being 0.66 and 0.33. This result is rather surprising since HSA contains only one tryptophan residue. In order to explain it, several hypothesis are considered.

In this preliminary work, we were concerned with the tryptophan fluorescence of human serum albumin.

Several works have already been published about spectra, quantum yield[1-4] and lifetime[5] of human serum albumin (HSA). No one has given a detailed account of the determination of the decay. This protein contains one tryptophan residue per molecule and one expects to find a single exponential decay.

On the contrary, we found a complex decay involving two decay times. This result will be discussed.

Experimental

Human serum albumin (HSA) was a 98 % pure crystaline sample purchased from Serva (Heidelberg). Urea was "Zur analyse" grade (E. Merck AG, Darmstadt, Germany) and dl-tryptophan was "puriss" grade (Fluka AG. Buch SG., Switzerland).

Fatty acids were removed from HSA by the charcoal treatment method of Chen at acid pH[7]. The activated charcoal used for that purpose (Darco G 60, Fluka AG. Buch SG.) was washed with distilled water, filtered, and dried prior to use.

The concentration of the untreated HSA solution used in this study was 1 mg/ml.

Absorption and fluorescence emission spectra were measured with a Cary-14 recording spectrometer and a Jobin Yvon spectrofluorimeter, respectively.

Relative fluorescence quantum yields were determined by the method of Parker and Rees[15] using dl-tryptophan in neutral aqueous solution as reference.

The nanosecond flash apparatus and the experimental procedures used for the determination of fluorescence lifetimes have been previously described[7,8]. A filter Schott WG 320, which eliminates, all the scattered light, was placed on the front of the photomultiplier.

Samples were maintained at a constant temperature of 20°C during all fluorescence measurements.

Results

It has been reported by Chen[4] that HSA samples sometimes contain impurities which can be removed by charcoal at neutral pH and that acid-charcoal treatment was an effective procedure for defatting serum albumin in addition to removing other impurities which may be present. In view of those findings, it was deemed necessary to make fluorescence measurements with both untreated and charcoal treated HSA samples.

According to Chen[4] the fluorescence emission spectra of HSA samples were either unchanged or showed considerably more tryptophan emission after charcoal treatment. In contradistinction, the exact opposite effect was observed in the present studies, i.e. the fluorescence emission spectra of the treated sample showed a lesser contribution from the single tryptophan residue. This is clearly evident from the data given in Table II. For excitation at 295 nm where only the tryptophan residue was excited, the

Table I : Relative fluorescence quantum yields and wavelength of maximum emission of human serum albumin at 20°C.

λ_{exc} (nm)	λ at F^{a}_{max} (nm)			Q^{b}_{rel}		
	untreated	Treated	untreated in 8M urea	untreated	Treated	untreated in 8M urea
295	343	342	356	1.07	0.86	1.39
290	341	340	354	0.88	0.56	0.85
280	339	337	350	0.69	0.44	0.46
270	339	337	350	0.65	0.40	0.42

[a] Estimated precision is ± 2 nm. [b] Quantum yields are relative to dl-tryptophan in neutral aqueous solution. Estimated precision is ± 10 %.

Table II : Fluorescence lifetimes of human serum albumin at 20°C.

pH	Untreated HSA					Treated HSA				
	A_1	τ_1 (nsec)	A_2	τ_2 (nsec)	$\langle\tau\rangle^{*}$ (nsec)	A_1	τ_1 (nsec)	A_2	τ_2 (nsec)	$\langle\tau\rangle$ (nsec)
5.5	0.66	3.3	0.34	7.8	4.8	0.71	3.9	0.29	7.9	5.1
3.3	0.78	2.5	0.22	7.1	3.5	0.75	3.6	0.25	8.0	4.7
5.5 (8M urea)	0.66	3.1	0.34	7.8	4.7					

* $\langle\tau\rangle = A_1 \tau_1 + A_2 \tau_2$. Wavelength of excitation was 295 nm in each case. Estimated precision of lifetimes is ± 0.2 nsec.

wavelength of the fluorescence maximum was essentially unchanged but at the same time the relative quantum yield of the untreated sample was greater than that of the treated sample. For excitation at 270 or 280 nm where both the tyrosine and tryptophan residues are excited, the fluorescence maximum of the charcoal treated sample was shifted slightly to shorter wavelengths indicating a greater contribution of the tyrosine fluorescence[2]. At each wavelength of excitation used in the present studies, the relative quantum yield of the

untreated sample was greater than that of treated HSA. These results conclusively show that the effect of charcoal treatment of HSA was to decrease the fluorescence due to the tryptophan residue.

The fluorescence decay of both treated and untreated HSA were measured using a nanosecond flash apparatus and the results of the convolution analysis of decay-time curves are summarized in Table II. The most noteworthy result of these measurements was that the HSA fluorescence did not decay as a single exponential as one would normally expect, but actually involved two distinctly different lifetimes (Fig. 1). The possibility that the second lifetime may be due to the tyrosine residues may be eliminated because the data given in Table II were obtained for fluorescence decay with excitation at 295 nm where the absorption of tyrosine is negligible.

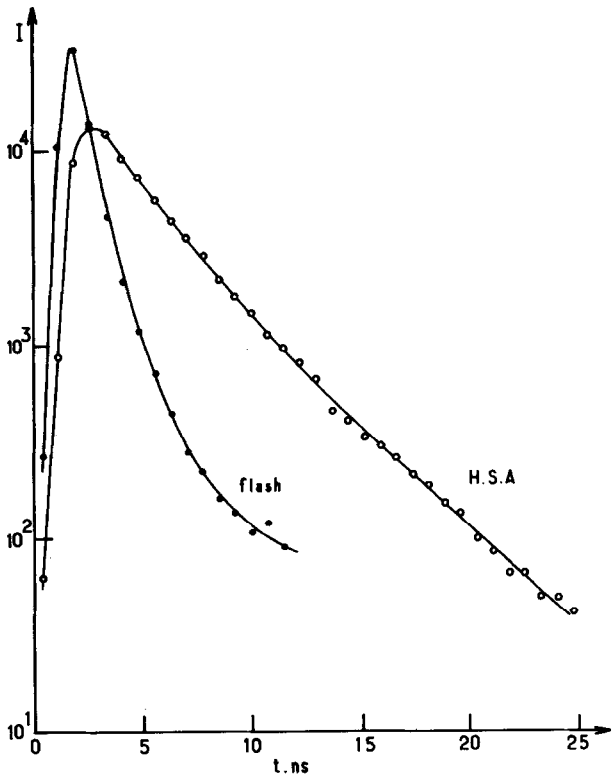

Fig. 1 : Fluorescence decay of human serum albumin λ_{exc} = 295 nm. The emission is viewed through a WG 320 Schott filter.

Lastly, the data given in columns 4 and 7 of Table I and the last line of Table II summarize the effect of 8 M urea upon the fluorescence of untreated HSA at pH 5.5. The previously reported shift of the fluorescence maximum of HSA in 8 M urea to that of tryptophan in neutral aqueous solution[1] was also observed in this study (fluorescence maximum of tryptophan was 355 nm). When both tryptophan and tyrosine were excited Qrel was lowered in 8 M urea, as it has been already reported[6]. This is interpreted as a decrease of the transfer from tyrosine to tryptophan in the unfolded molecule. When only tryptophan is excited (λex = 295 nm) Qrel was higher than its corresponding value in aqueous solution. This comes probably from specific interaction of urea with the accessible tryptophan residue. It is known that the quantum yield of free tryptophan in solution is enhanced by the presence of urea[2]. In addition it can be seen in Table II that only the short lifetime τ_1 was slightly affected by 8 M urea.

Discussion

At first sight it is rather surprising to find two fluorescence decay times for the single tryptophan residue of HSA. The presence of two different lifetimes is also suggested by some recent studies on fluorescence quenching[18]. One may think, the samples studied contain some tryptophan aminoacid bound to the protein as an impurity, since serum albumin can bind strongly that aminoacid[9]. The complex decay observed would be the result of the superposition of the emission of the bound tryptophan and of the emission of the residue incorporated in the peptide chain. Charcoal treatment would eliminate such bound molecules and would affect strongly the shape of the decay. In fact, the decays at pH 5.5 are very similar for the treated and untreated protein, and one must find another interpretation.

The complex decay might come from some heterogeneity of HSA. At least three kinds of heterogeneity have been reported to occur in HSA :

1) A fraction of the molecules have titratable sulphydryl groups (mercaptalbumin)[10].

2) Dimers and higher polymers are generally present[11].

3) Finally, following Foster[12], serum albumins have a microheterogeneity corresponding to fractions of different solubilities.

Besides these chemical explanations, one might also think of two different physical interpretations.

The first one will only concern interactions in the ground state. One can assume that the molecules with different conformations are in equilibrium in the solution and that in each conformation, the tryptophan residue has a different environment[14]. Then the fluorescence will be the superposition of different emissions corresponding to the different conformations.

The second interpretation involves the interactions in the excited state of the indole ring. It has been shown that the interactions of indole and tryptophan, with polar solvents, are different in the ground state and in the excited state. The solvent molecules surrounding the excited molecules are initially in the configuration which prevails in the ground state (Franck-Condon principle).

Then a relaxation occurs during the exciting state, leading to a configuration of energy minimum. These processes are : either the reorientation of solvent molecules[15] or the exciplexes formation[16]. A non exponential decay may result from these phenomena[8,19]. The study of the emission spectra at various temperatures has shown that relaxation processes occur also with the tryptophan residues in proteins[17].

We are now proceeding with experiments in order to determine which explanation must be finally retained.

References

1 - Teale, F.W.J., Biochem. J. 76, 381 (1960).
2 - Vladimirov, Yu. A. and Zimina, G.M., Biokhimiya, 30 1105 (1965).
3 - Chen, R.F., Biochimica Biophysica Acta, 120, 169 (1966).
4 - Chen, R.F., J. Biol. Chem. 242, 173 (1967).
5 - Chen, R.F., Varek, G.G., Alexander, N., Science, 156, 949 (1967).
6 - Weber, G., Biochem. J. 79, 29P (1961).
7 - Wahl, Ph., Biochimica Biophysica Acta, 175, 55 (1969).
8 - De Lauder, W.B. and Wahl, Ph., Biochemistry, 9, 2750 (1970).
9 - McMenamy, R.H., J. Biol. Chem., 240, 4235 (1965).
10 - Hughes, W.L. Jr., J. Am. Chem. Soc., 69, 1836 (1947).
11 - King, T.P., Yphantis, D.A., and Craig, L.L., J. Am. Soc. 82, 3355, (1960).
12 - Sogami, M. and Foster, J.F., Biochemistry, 7, 2172 (1968).
13 - Parker, C.A. and Rees, W.T., Analyst., 85, 587 (1960).
14 - Mazhul', V.M., Chernitskii, and Konev, S.V., Biofizika 15, 5 (1970).
15 - Eisinger, J. and Navon, G., J. Chem. Phys. 50 , 2069 (1969).
16 - Walker, M.S., Bedmar Th., and Lumry, R., J. Chem. Phys., 47, 1020 (1967).
17 - Longworth, J.W. and Battista, M.D.C., Photochem. Photobiol. 11, 875 (1970).
18 - Teale, F.W.J., personal communication.
19 - Bakhshiev, N.G., Mazurenko Yu T. and Peterskaya I.V., Opt. Spectrosk. 21, 550 (1966).