Biophysical Chemistry 19 (1984) 337–344
Elsevier

BPC 00854

EFFECT OF LIGAND BINDING AND CONFORMATIONAL CHANGES IN PROTEINS ON OXYGEN QUENCHING AND FLUORESCENCE DEPOLARIZATION OF TRYPTOPHAN RESIDUES

Badri P. MALIWAL and Joseph R. LAKOWICZ *

University of Maryland, School of Medicine, Department of Biological Chemistry, 660 W. Redwood Street, Baltimore, MD 21201, U.S.A.

Received 22nd November 1983
Revised manuscript received 23rd December 1983
Accepted 4th January 1984

Key words: Fluorescence depolarization; Ligand binding; Oxygen quenching; Tryptophan fluorescence

The rotational freedom of tryptophan residues in protein-ligand complexes was studied by measuring steady-state fluorescence anisotropies under conditions of oxygen quenching. There was a decrease in the oxygen bimolecular quenching constant upon complexation of trypsin and α-chymotrypsin with proteinaceous trypsin inhibitors, of lysozyme with *N*-acetylglucosamine (NAG) and di(*N*-acetyl-D-glucosamine) ($(NAG)_2$) and of hexokinase with glucose. Binding of the bisubstrate analogue *N*-phosphonacetyl-L-aspartate (PALA) to aspartate transcarbamylase (ATCase) and binding of biotin to avidin resulted in increased oxygen quenching constants. The tryptophan of human serum albumin (HSA) in the F state was more accessible to oxygen quenching than that in the N state. With the exception of ATCase, the presence of subnanosecond motions of the tryptophan residues in all the proteins is suggested by the short apparent correlation times for fluorescence depolarization and by the low apparent anisotropies obtained by extrapolation to a lifetime of zero. Complex formation evidently resulted in more rigid structures in the case of trypsin, α-chymotrypsin and lysozyme. The effects of glucose binding on hexokinase were not significant. Binding of biotin to avidin resulted in a shorter correlation time for the tryptophan residues. The N → F transition in HSA resulted in a more rigid environment for the tryptophan residue. Overall, these changes in the dynamics of the protein matrix and motional freedom of tryptophan residues due to complex formation and subsequent conformational changes are in the same direction as those observed by other techniques, especially hydrogen exchange. Significantly, the effects of complex formation on protein dynamics are variable. Among the limited number of cases we examined, the effects of complex formation were to increase, decrease or leave unchanged the apparent dynamics of the protein matrix.

1. Introduction

There is considerable theoretical and experimental evidence that proteins have significant internal freedom and that a wide variety of motions can occur. The flexibility of proteins probably plays an important role in their biological functions, such as in enzyme catalysis, binding of ligands, the multiple functions of antibodies, and in the assembly of structures [1–4]. Fluorescence spectroscopy is useful for probing events which occur during the excited-state lifetimes of the fluorophores, typically on the nanosecond time scale. Initially, protein flexibility on a nanosecond time scale was suggested by diffusion of quenchers like oxygen and acrylamide through the protein matrix [5–9]. Subsequently, rotational freedom for tryp-

* To whom correspondence and reprint requests should be addressed.

Abbreviations: BPTI, bovine pancreatic trypsin inhibitor; LBTI, lima bean trypsin inhibitor; NAG, *N*-acetyl-D-glucosamine; $(NAG)_2$, di(*N*-acetyl-D-glucosamine); PALA, *N*-phosphonacetyl-L-aspartate; ATCase, aspartate transcarbamylase; HSA, human serum albumin; ϕ_A, apparent correlation time.

tophan residues in proteins was also indicated by time-resolved decays of emission anisotropy [10–12], from steady-state measurements of anisotropy as a function of temperature and viscosity [13] and by lifetime-resolved anisotropies. In the latter method the lifetime of the fluorophore is reduced by collisional quenching, and the steady-state anisotropy is measured as a function of lifetime [6,7,14,15]. In the present communication we describe the diffusion of oxygen through the protein matrix and the rotational freedom of tryptophan residues in several proteins and their ligand complexes. Binding of ligands such as substrates, inhibitors, or activators results in significant conformational changes in all these proteins. We wished to examine the effects of these conformational changes on the dynamics of protein matrix. The proteins and ligands we studied were trypsin and α-chymotrypsin and trypsin inhibitors (BPTI and LBTI); lysozyme and NAG and its dimer; hexokinase and glucose; avidin and biotin; ATCase and its substrate analog PALA; and HSA at pH 5.5 (N form) and at pH 3.3 (F form).

2. Materials and methods

BPTI was a gift from Dr. C. Woodward (University of Minnesota). LBTI, lysozyme and HSA were purchased from Worthington Chemicals. α-Chymotrypsin, trypsin, hexokinase, avidin, biotin, glucose and NAG were purchased from Sigma Chemical Co. ATCase and PALA were gifts from Dr. N. Allewell (Weselyan University) and $(NAG)_2$ was a gift from Dr. J. Rupley (University of Arizona).

2.1. Fluorescence spectral measurements

Fluorescence lifetimes were measured by the phase shift method using a modulation frequency of 30 MHz [16] and the reference compound 2,5-diphenyl-1,3,4-oxadiazole with a lifetime of 1.2 ns [17]. The excitation wavelength was 295 nm and emission was observed through a 344 nm interference filter of 10 nm bandpass [6]. For anisotropy measurements the proteins were excited at 300 nm (slits 8 and 2 nm) and emission was observed at 344 nm (slits 8 and 8 nm). The first and second slit widths are the entrance and exit slit of the monochromator, respectively. Details of oxygen quenching of fluorescence and anisotropy measurements under these conditions were described previously [5,6].

3. Theory

Information about the rotational motions of fluorophores can be derived from measurements of the fluorescence anisotropy. The anisotropy is defined as

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}} \tag{1}$$

where $I_{\parallel}$ and $I_{\perp}$ are vertical and horizontal polarized components of emission from a sample which is excited with vertically polarized light. The time-dependent decay of anisotropy depends on the rotational diffusion of the fluorophore. For a completely anistropic molecule the decay of anisotropy is the sum of five exponentials.

$$r(t) = \sum_{i=1}^{5} r_0 e^{-t/\phi_i}. \tag{2}$$

where r_0 is the anisotropy in the absence of rotational diffusion and ϕ_i the rotational correlation times which are dependent upon the diffusion coefficients around each molecular axis [18]. For practical purposes this decay is well approximated by only three terms. In our analysis a simpler model will be used. We assume that the overall rotational displacement of the protein can be described with a single correlation time ϕ_P, and that segmental motions of the tryptophan residue within the protein matrix are adequately described by a single correlation time ϕ_T. That is, the rotational displacement of tryptophan residue in a protein is treated as a superimposition of the overall protein rotation and of the motions of the residue within the protein matrix [10]. Depending upon the motional freedom of the residue within the protein matrix some fraction (α) of the total anisotropy (r_0) is lost due to segmental motions. The remaining anisotropy ($1 - \alpha$) decays as a result of

rotational diffusion of the protein. Hence,

$$r(t) = r_0\left[\alpha e^{-t/\phi_T} + (1-\alpha)\right] e^{-t/\phi_P}. \quad (3)$$

We recognize that the decay of anisotropy for flexible molecules is more complex [19]. However, practical considerations have limited our analysis and those described by other researchers to these or similar expressions. The steady-state anisotropies at various lifetimes ($r(\tau)$) are given by the average of $r(t)$ over the total decay of fluorescence intensity. For a single exponential decay of intensity ($e^{-t/\tau}$) the lifetime-dependent anisotropy is given by

$$r(\tau) = \frac{\int_0^\infty r(t) e^{-t/\tau} dt}{\int_0^\infty e^{-t/\tau} dt} \quad (4)$$

Substitution of eq. 3 into eq. 4 yields

$$r(\tau) = \frac{\alpha r_0}{1 + \left(\frac{1}{\phi_T} + \frac{1}{\phi_P}\right)\tau} + \frac{(1-\alpha) r_0}{1 + \frac{\tau}{\phi_P}} \quad (5)$$

Eq. 5 can be used to estimate the form of the anisotropy decay and hence the dynamic properties of the protein matrix surrounding the fluorophore. A plot of r^{-1} vs. τ yields r_0^{-1} as the y-intercept and $(r_0\phi_A)^{-1}$ as the slope. Hence, both r_0 and an apparent rotational correlation time can be estimated. We refer to the anisotropy value obtained by extrapolation to $\tau = 0$ as $r(0)$. Eq. 5 predicts that if the fluorophore is rigidly held by the protein matrix, the observed correlation time will be the protein correlation time (ϕ_P) and the extrapolated value of $r(0)$ will be equal to r_0. The presence of segmental motions for the tryptophan residues will be revealed by the extrapolated value of $r(0)$ being lower than r_0 and/or by an apparent correlation time (ϕ_A) which is smaller than that expected for rotational diffusion of the protein. If the motions are on the picosecond time scale then the depolarizing effects of these motions will be completed even for our shortest quenched lifetimes ($\simeq 0.8$ ns). Then, the extrapolated $r(0)$ value will be smaller than r_0 but the observed ϕ_P will be equal to that expected for the protein molecule. If the segmental motions are on the nanosecond time scale the difference between $r(0)$ and r_0 may not be observable. However, the segmental motions are usually revealed by values of ϕ_A which are smaller than expected for overall protein rotation. Detailed model calculations were presented in earlier publications [6,7].

We measured steady-state anisotropies as the fluorescence lifetimes were decreased by oxygen quenching. These lifetimes (τ) can be calculated from

$$\tau = \frac{\tau_0}{1 + K[O_2]} \quad (6)$$

where τ_0 and τ are the lifetimes in the absence and presence of oxygen, respectively, and K the collisional Stern-Volmer quenching constant. The validity of eq. 6 for quenching of protein fluorescence by oxygen has been previously demonstrated [5]. Another useful result from the quenching experiments is the bimolecular quenching constant k_q. This constant reflects the collisional frequency of fluorophore with oxygen and thus the rate of oxygen diffusion through the protein matrix. This supposition has been tested rigorously in the elegant studies of Calhoun et al. [20] and found to be valid. We obtained measured values of k_q using the Stern-Volmer equation,

$$\frac{F_0}{F} = 1 + k_q\tau_0[O_2] \quad (7)$$

where F_0 and F are the fluorescence intensities in the absence and presence of oxygen, respectively, and τ_0 the lifetime in the absence of quenching.

4. Results

4.1. Fluorescence spectra and lifetimes

There was a blue shift in the fluorescence emission upon binding of trypsin inhibitors to trypsin and α-chymotrypsin, of glucosamines to lysozyme and of biotin to avidin. The fluorescence emission of ATCase and hexokinase did not change due to ligand binding. These results are similar to those documented in the literature. The only significant changes in lifetime were in the cases of HSA (N → F transition; 15% decrease), avidin (100%

Table 1

Unquenched fluorescence lifetimes and oxygen quenching constants for proteins and protein-ligand complexes

(a) 0.001 M HCl, pH 3.0; (b) 0.1 M phosphate buffer, pH 7.5; (c) 0.05 M acetate buffer, 0.05 M NaCl, pH 5.2; (d) 0.01 M Tris-HCl, 0.1 M NaCl, pH 8.0; (e) 0.02 M Tris-HCl, 0.2 M NaCl, pH 8.5; (f) 0.05 M phosphate buffer, pH 7.5; (g) e containing 10 mM $MgCl_2$; (h) 0.1 M NaCl, pH 5.5; (i) 0.1 M NaCl, pH 3.3; (j) 0.04 M phosphate, 2 mM EDTA, pH 7.5.

Protein/ligand	Temperature (°C)	τ_0 (ns) *	K (M^{-1})	k_q ($\times 10^{-10}$) (M^{-1} s^{-1})	Δk_0 (%)
α-Chymotrypsin (a)	25	2.0	4.6	0.24	
+ LBTI (b)	25	2.8	4.6	0.16	−30
Trypsin (a, c)	25	2.0	12.3	0.61	
+ BPTI (d)	25	2.0	9.2	0.46	−25
+ LBTI (c)	25	2.0	10.7	0.53	−13
Avidin (e)	25	1.8	4.9	0.27	
+ biotin	25	0.8	3.0	0.37	+31
Lysozyme (f)	25	1.8	7.8	0.44	
+ NAG	25	1.8	5.9	0.32	−27
+ $(NAG)_2$	25	1.8	5.2	0.28	−36
Hexokinase (g)	25	3.5	13.0	0.37	
+ glucose	25	3.4	10.9	0.32	−14
HSA N form (h)	25	3.8	11.1	0.29	
F form (i)	25	3.3	19.3	0.58	+100
ATCase (j)	25	1.8	9.6	0.52	
+ PALA (10 mol/mol ATCase)	25	2.0	11.2	0.57	+9

* The uncertainty is about ±0.2 ns. For comparative purposes the relative uncertainty is about ±0.1 ns.

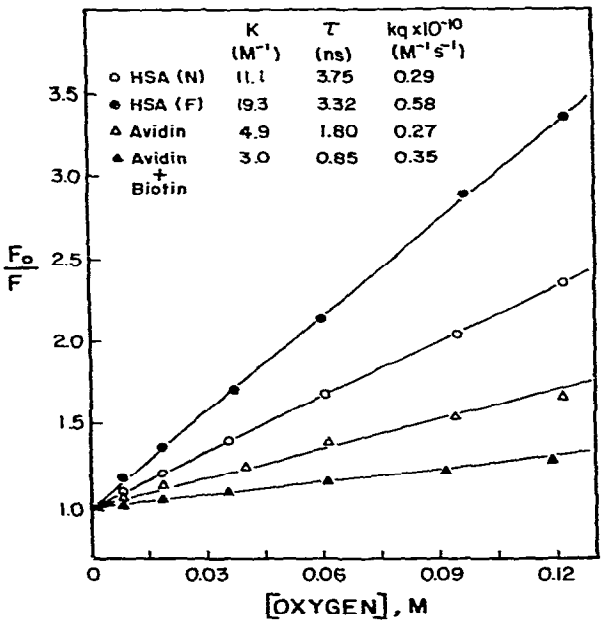

Fig. 1. Stern-Volmer plot for oxygen quenching of HSA (N and F forms), avidin and the avidin-biotin complex.

decrease) and α-chymotrypsin (50% increase). The Stern-Volmer quenching constants, lifetimes and bimolecular quenching constants are summarized in table 1 and some examples are shown in fig. 1. The bimolecular quenching constants ranged from 0.6×10^{10} M^{-1} s^{-1} for trypsin to 0.24×10^{10} M^{-1} s^{-1} for α-chymotrypsin. These values are about 50–20% of the diffusion-controlled limits and are within the range of values observed previously for these and other proteins [5–6]. The binding of protein inhibitors to trypsin and α-chymotrypsin, glucosamines to lysozyme and glucose to hexokinase resulted in decreased bimolecular quenching constants. There was a small increase in the bimolecular quenching constant due to the interaction of PALA with ATCase, and biotin

with avidin, and a somewhat larger increase for the F form of HSA as compared to the N form. There is some uncertainty regarding k_q for the avidin-biotin complex. The lifetime of the complex is about 0.8 ns and the extent of quenching is only 25%. Both values are small and the relative error of the measurement is larger. A small error in either τ_0 or the extent of quenching will result in a significant change in the calculated value of k_q.

4.2. Lifetime-resolved anisotropies and correlation times

The apparent limiting anisotropies and correlation times are given in table 2 and fig. 2. We could not determine the rotational correlation time of ATCase (M_r 310000) since there was no depolarization due to its large correlation time ($\phi_P = 160$ ns) and lack of segmental motions ($r(0) = 0.27$). In all other cases the apparent correlation times (ϕ_A) are smaller than those expected for proteins of respective sizes (ϕ_P), and $r(0)$ is smaller than the fundamental anisotropy for the tryptophan ($r_0 =$ 0.26) [6]. These results suggest the presence of rotational freedom for at least some tryptophan residues. It should be noted that all the proteins under study, except HSA, are multitryptophan proteins and that the calculated values of ϕ_P are only estimates of the actual protein correlation times. Nonetheless, a comparison of the ϕ_A and ϕ_P values from table 2 reveals some cases where ϕ_A is substantially shorter than ϕ_P (trypsin, avidin and lysozyme). In these cases, the presence of substantial segmental motions seems fairly certain.

The binding of trypsin inhibitors to trypsin and α-chymotrypsin, of glucosamine to lysozyme, and the N → F transition of HSA resulted in an increase in both the apparent limiting anisotropy, $r(0)$, and the correlation times, indicating a decrease in the motional freedom of the tryptophan residues. There was no significant effect of the binding of PALA to ATCase on $r(0)$ and of glucose binding to hexokinase on either $r(0)$ or ϕ_A. Surprisingly, there was a decrease in both $r(0)$ and ϕ_A due to binding of biotin to avidin, which suggests an increase in the motional freedom of tryptophan residues in the biotin-avidin complex.

Table 2

Correlation times and $r(0)$ values for tryptophan residues in proteins and protein-ligand complexes at 25°C

Protein/ligand	ϕ_A (ns) [a]	ϕ_P (ns) [b]	$r(0)$	ϕ_P/ϕ_A	$r_0/r(0)$
α-Chymotrypsin	7.1	12.7	0.179	1.8	1.45
+LBTI	9.7	17.5	0.209	1.8	1.24
Trypsin	5.1	12.6	0.250	2.5	1.04
+LBTI	9.3	17.4	0.252	1.9	1.03
+BPTI	7.6	16.1	0.252	2.1	1.03
Avidin	6.3	36	0.147	5.7	1.77
+biotin	3.0		0.135	12	1.92
Lysozyme	3.9	7.6	0.230	1.9	1.13
+NAG	5.0		0.246	1.5	1.06
+$(NAG)_2$	5.1		0.243	1.5	1.08
Hexokinase	17.5	27	0.238	1.5	1.09
+glucose	17.5		0.238	1.5	1.09
HSA N form	21.5	34	0.261	1.6	1.00
F form	27.4		0.260	1.2	1.00
ATCase	–	160	0.270	–	0.96
+PALA	–		0.270	–	0.96

[a] Apparent rotational correlation time, as obtained from the plots of $[r(\tau)]^{-1}$ vs. τ.

[b] Rotational correlation times calculated for overall rotation of the protein using $\phi_P = (\eta V/RT)f$, where η is the viscosity, V the volume and f a factor, taken as equal to 2, which accounts for the hydration and asymmetry of the protein [6].

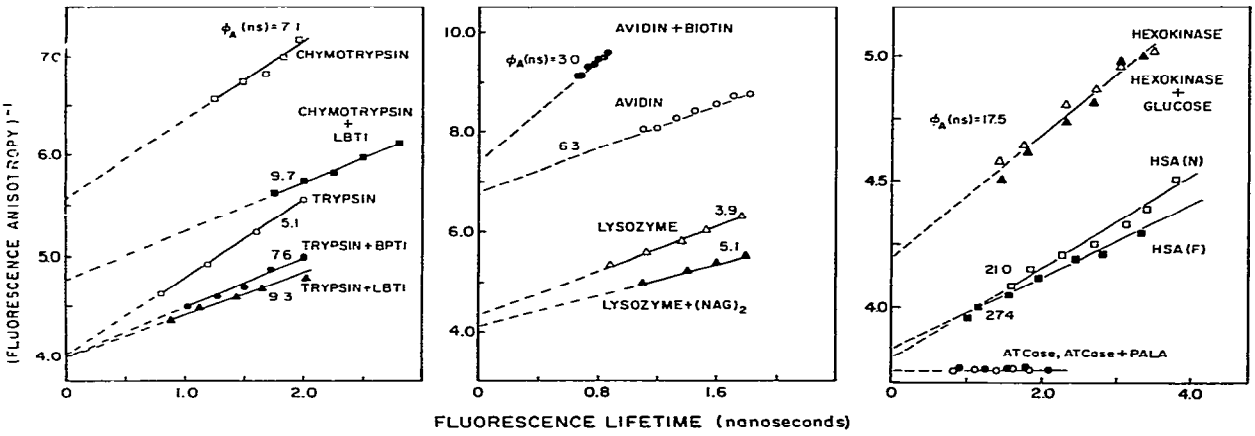

Fig. 2. Lifetime-resolved anisotropies of varous protein-ligand complexes.

5. Discussion

Binding of ligands results in extensive changes in the protein molecules, effects which propagate far beyond the binding site in all the protein-ligand complexes we examined. The decrease in the bimolecular quenching constants and the increase in the apparent correlation time and $r(0)$ in the case of binding of trypsin inhibitors to trypsin and α-chymotrypsin, and for binding of NAG and $(NAG)_2$ to lysozyme indicate that complex formation results in a more rigid protein structure and less accessible tryptophan residues. These results are in accord with the decreased rates of hydrogen exchange observed for complexed trypsin [21] and lysozyme [28] and a dramatic decrease in accessibility of tryptophan residues to *N*-bromosuccinamide in the case of trypsin [22].

Substrate binding often results in large conformational changes of most kinases as is shown by X-ray crystallography. The hexokinase molecule is made up of two domains which are linked by a flexible hinge [23]. Binding of glucose brings these two domains closer. Our results to some extent support this model. We also found a decrease in bimolecular quenching constant, as might be expected for a more compact structure. However, glucose binding did not affect the segmental mobility of tryptophan residues. Feldman and coworkers [24,25] using various quenchers showed that tryptophans in hexokinase can be classified into three groups of emitters: the two surface residues, the buried residue, and a tryptophan residue present in the binding site which is quenched by glucose. Our results indicate that despite the significant rearrangement in tertiary and local structure seen by X-ray crystallography the segmental motions in the regions where emitting tryptophan residues are located are not significantly altered by the binding of glucose.

The fluorescence of lysozyme may originate from the three classes of tryptophan residues. Two residues, Trp-62 and Trp-108, which are present in the substrate-binding site, contribute about 80–85% of the fluorescence intensity, while the remaining 15–20% is contributed by three residues which are located in the hydrophobic interior of the protein [26–28]. We found that binding of both NAG and $(NAG)_2$ resulted in decreased segmental mobility of the tryptophan residues, and shielding of these residues from collisional quenching by oxygen. These results are in agreement with the significant decrease in the hydrogen-exchange rate of the lysozyme molecule which occurs upon complex formation [29]. A decrease in motional freedom and shielding from quenching of the tryptophan residues which are part of the binding site are also expected from the multiple hydrogen

bonds observed in the X-ray structure between the sugar residues and various amino acids.

A variety of techniques suggest that binding of substrate and substrate analogs to ATCase causes significant reorganization in the quaternary and perhaps tertiary structure and promotes a more flexible state (R state) [31,32]. We observed an increase, though a small one, in the bimolecular quenching constant due to interaction of PALA, which suggests more accessible tryptophan residues. One possible explanation for a small effect may be that the tryptophan residues in ATCase are near the surface and exposed to solvent and as such are already highly accessible (k_q is about 50% of diffusion-controlled limits).

The transformation of albumin from the N to F state between pH 4.3 and 3.7 results in a variety of changes in the physical and chemical properties of the albumin molecule. There is some decrease in helicity and albumin in the F state has a larger hydrodynamic volume [33]. The N → F transition also affects the microenvironment of its tryptophan residue as seen by a decreased intensity, a blue shift of the emission maximum [33], and altered binding of insecticides to the site where tryptophan is present [34]. The tryptophan residue in the F state is more accessible to oxygen as indicated by an increase in k_q. However, the blue shift of the emission may indicate that the fluorophore becomes buried in a relatively nonpolar region of the protein interior, which is in apparent contradiction to the increased accessibility to oxygen. The tryptophan residue in the F state of HSA is still accessible to solvent perturbants of a size near 5 Å [36], which also suggests that it is not buried in the protein interior. The probable cause of this blue shift is an increased rigidity of the environment surrounding tryptophan [35] which is indicated by an increase in ϕ_A for the F form. A similar increase in ϕ_A during the N → F transition of bovine serum albumin was observed by Sogami et al. [36] using variation in viscosity and iodide quenching. Sogami et al. [36] also observed a parallel increase in ellipticity at 295–300 nm during the N → F transition. Increased asymmetry of the protein matrix could lead to more optical asymmetry and hence an increase in the circular dichroism.

Binding of biotin with avidin results in shielding of tryptophan residues from solvent (blue shift), a significant decrease in reaction with *N*-bromosuccinamide and a protein molecule greatly stabilized against denaturation by heat. Also, binding requires tryptophan residues as indicated by a large decrease in the affinity due to chemical modifications of the tryptophan residues [37]. Surprisingly, binding of biotin led to an increased bimolecular quenching constant and a decrease in $r(0)$ and ϕ_A, suggesting that at least some of the tryptophan residues in the avidin-biotin complex are more accessible and mobile. We do not have any explanation for this seemingly contradictory behavior, especially in view of the direct contacts between biotin and tryptophan residues and the lack of any significant change in secondary structure of complexed avidin [38].

In summary, we observed changes in both accessibility of tryptophan residues to oxygen and in their segmental motions upon binding of ligands to proteins. Most of the changes were in agreement with that predicted on the basis of other experimental data, especially hydrogen exchange. We must emphasize that all the proteins in the present investigation, except HSA, have multiple tryptophan residues; therefore, we observe an average effect which does not distinguish between the effects on individual tryptophan residues. Often, different tryptophan residues in a protein molecule have different quantum yields, and hence the averages will be dominated by the effects on those residues which fluoresce with higher quantum yields. We also note that oxygen, being small and nonpolar, penetrates protein matrices very effectively. The difference in quenching between exposed and completely buried residues is less than an order of magnitude [5,6,20]. This efficiency in penetrating the protein matrix will underemphasize the changes and it will be insensitive to small effects of the ligands. A larger quencher which can also penetrate the protein matrix is likely to be more discriminating and may provide a more effective view of the ligand-induced changes. Despite these uncertainties the present study reveals that quenching and lifetime-resolved anisotropies can be effectively used to study the effects of conformational changes on the dynamics of proteins. Some recent developments, such as

phase-sensitive detection to resolve heterogeneous populations of fluorophores [39], and the availability of continuously variable frequency phase fluorometers which can resolve small lifetime differences [40] and continuously improving resolution from pulse fluorometry [41] may make possible the resolution of heterogeneous fluorescence of proteins. This ability to resolve heterogeneous fluorescence from proteins will help in observing changes in the dynamics of individual residues.

Acknowledgements

This work was supported by Grants PCM 80-41320 and PCM 81-06910 from the National Science Foundation. This work was done during the tenure of an Established Investigatorship (to J.R.L.) from American Heart Association.

References

1 F.R.N. Gurd and T.M. Rothgeb, Adv. Protein Chem. 33 (1979) 73.
2 M. Karplus and J.A. McCammon, CRC Crit. Rev. Biochem. 9 (1981) 293.
3 R.J.P. Williams, Proc. R. Soc., B 200 (1978) 353.
4 G. Careri, P. Fasella and E. Gratton, Annu. Rev. Biophys. Bioeng. 8 (1979) 69.
5 J.R. Lakowicz and G. Weber, Biochemistry 12 (1973) 4171.
6 J.R. Lakowicz, B.P. Maliwal, H. Cherek and A. Balter, Biochemistry 22 (1983) 1741.
7 J.R. Lakowicz and B.P. Maliwal, J. Biol. Chem. 258 (1983) 4794.
8 M.R. Eftink and C.A. Ghiron, Biochemistry 15 (1976) 672.
9 M.R. Eftink and C.A. Ghiron, Anal. Biochem. 114 (1981) 199.
10 P.H. Wahl, in: Biochemical fluorescence: Concepts, eds. R.F. Chen and H. Edelhoch, vol. 1 (Dekker, New York, 1976) p. 1.
11 I. Monro, I. Pecht and L. Stryer, Proc. Natl. Acad. Sci. U.S.A. 76 (1979) 56.
12 J.B.A. Ross, K.W. Rousslang and L. Brand, Biochemistry 20 (1981) 4361.
13 S.R. Anderson, M. Brumori and G. Weber, Biochemistry 9 (1970) 4723.
14 F.W.J. Teale and R.A. Bradley, Biochem. J. 116 (1970) 341.
15 J.R. Lakowicz and G. Weber, Biophys. J. 32 (1980) 591.
16 R.D. Spencer and G. Weber, Ann. N.Y. Acad. Sci. 158 (1969) 361.
17 J.R. Lakowicz, H. Cherek and A. Balter, J. Biochem. Biophys. Methods 5 (1981) 131.
18 G.G. Belford, R.L. Belford and G. Weber, Proc. Natl. Acad. Sci. U.S.A. 69 (1972) 1392.
19 S.C. Harvey and H.C. Cheung, Biopolymers 19 (1980) 913.
20 D.B. Calhoun, J.M. Vanderkooi, G.V. Woodrow and S.W. Englander, Biochemistry 22 (1983) 1526.
21 C.K. Woodward, J. Mol. Biol. 111 (1977) 509.
22 R.F. Steiner, Biochemistry 5 (1966) 1964.
23 C.M. Anderson, F.H. Zucker and T.A. Steitz, Science 204 (1979) 375.
24 I. Feldman and G.E. Norton, Biochim. Biophys. Acta 615 (1980) 132.
25 I. Feldman, T.M. Fame and D.A. Goldstein, Biochim. Biophys. Acta 703 (1982) 34.
26 T. Imoto, L.S. Forster, J.A. Rupley and F. Tanaka, Proc. Natl. Acad. Sci. U.S.A. 69 (1972) 1151.
27 C. Formoso and L.S. Forster, J. Biol. Chem. 250 (1975) 3738.
28 T. Imoto, L.N. Johnson, A.C.T. North, D.C. Phillips and J.A. Rupley, in: The enzymes, ed. P.D. Boyer, vol. 7 (Academic Press, New York, 1972) p. 665.
29 R.R. Wickett, G.J. Ide and A. Rosenberg, Biochemistry 13 (1974) 3273.
30 G.J. Howlett, M.N. Blackburn, J.G. Compton and H.K. Schachman, Biochemistry 17, (1977) 5091.
31 E.R. Kantrowitz, S.C. Pastra-Landis and W.N. Lipscomb, Trends Biochem. Sci. 5 (1980) 150.
32 M. Lennick and N. Allewell, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 6759.
33 J.F. Forster, in: Albumin structure, function and uses, eds. V.M. Rosenoer, M. Oratz and M.A. Rothschild (Pergamon Press, New York, 1977) p. 53.
34 B.P. Maliwal and F.E. Guthrie, Mol. Pharmacol. 20 (1981) 138.
35 J.R. Lakowicz and A. Balter, Photochem. Photobiol. 36 (1982) 125.
36 M. Sogami, S. Ara, S. Nagaoka and H. Inouye, Int. J. Peptide Protein Res. 19 (1982) 263.
37 M.N. Green, Adv. Protein Chem. 29 (1975) 85.
38 R.B. Honzatko and R.W. Williams, Biochemistry 21 (1982) 6201.
39 J.R. Lakowicz and H. Cherek, J. Biol. Chem. 256 (1981) 6348.
40 J.R. Lakowicz, E. Gratton, B. Maliwal, G. Laczko, H. Cherek and M. Limkeman, Biophys. J. (1984) in the press.
41 J.R. Knuston, D.G. Walbridge and L. Brand, Biochemistry 21 (1982) 4671.