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Picosecond fluorescence decay of lens protein γ-II crystallin

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Abstract

The fluorescence decay of tryptophan residues in the bovine lens protein γ-II crystallin has been measured in aqueous buffer solutions. Results were obtained as a function of emission wavelength, temperature, dissolved oxygen, and denaturing solvent. The protein displayed complex fluorescence decay which fit a biexponential model with a long component (ns) and a short component (few hundred ps). Measured fluorescence quantum yields data for γ-II crystallin allowed calculation of radiative and non-radiative rate constants. The radiative rate constant was consistent with that observed in other indole derivatives, while the nonradiative rate constant was quite large and accounted for the short lifetime in γ-II. The temperature dependence of the non-radiative decay in γ-II crystallin yielded a small activation energy of only 1–2 kcal/mol, compared to 4 kcal/mol for the reference compound NATA whose barrier is known to derive from the rotamer model.

Keywords: Fluorescence lifetimes; Lens proteins; Crystallins; Tryptophan; Non-radiative decay

1. Introduction

The decay of protein fluorescence is sensitive to changes in the environments of emitting fluorophores [1]. Thus, fluorescent probes near the binding sites of enzymes can serve as reporter groups for substrate or cofactor binding [2]. Similarly, changes in protein conformation can affect the emission properties of fluorescent tryptophan residues [3]. Examples involving dynamics of local and global motions in proteins have also been reported [4].

The crystallin proteins are responsible for maintaining the transparency of the ocular lens [5]. Hence, the light absorption [6], emission [7–10], Rayleigh [11,12] and Raman scattering [13] properties of the crystallins are important. Changes in the conformation and/or state of aggregation of the crystallins may accompany cataracts [14–17] resulting in loss of lens transparency. Complete X-ray structures are available for a few members of the gamma crystallin family [18,19], including bovine γ-II crystallin.

Photochemical reactions of lens crystallin proteins are of potential importance in the etiology of human cataracts and studies of the effect of

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UV radiation on whole lenses and lens protein fractions have appeared [20–27]. The aromatic amino acid tryptophan seems to play a key role in some types of lens photodamage [20,27]. Photochemical reactions of amino acids and proteins, may originate from excited singlet states, excited triplet states, and photoionized states. Photochemical reactions which originate from the lowest excited singlet states (S_1) of proteins compete in time with fluorescence emission. Hence measurement of fluorescence decay provides information on the rates of excited singlet state processes, including photochemical reactions. It has been suggested that protein photochemistry may stem from a photoionized state of tryptophan, but there has been controversy over whether ionization originates from the same precursor state as fluorescence or from a "pre-fluorescent state" [28–30]. Fluorescence lifetime measurements can aid in answering such questions.

In the present paper, we report measurements of the nanosecond and picosecond decay components of tryptophan residues in the bovine lens protein γ-II crystallin, including effects of temperature, dissolved oxygen, and denaturing solvent. None of these properties have been reported previously.

2. Materials and methods

Separation of the γ-crystallin was performed according to Björk [31]. Four lenses were homogenized with 10 mL of buffer (Tris-Cl 0.05 *M*, pH 7.2, and 5 m*M* mercaptoethanol) and centrifuged. The supernatant was applied to a 5.0 × 60.0 cm glass column (#XK50/60, Pharmacia LKB Biotechnology Inc., Piscataway, NJ) packed with Sephadex G-75 (also from Pharmacia) and eluted with 1300 mL of the above Tris buffer at a rate of 0.8 mL/min. The separations were achieved in a 4°C cold box. After separation, the crystallin was lyophilized and stored at −5°C until needed. The collected γ-crystallin was pooled and dialyzed in Spectrapor 6000–8000 pore size membranes (Fisher Chemical Co.) prior to lyophilization.

Tryptophan monomer and *N*-acetyltryptophan amide (NATA) were used as standards for comparison with the lens crystallin protein. These were obtained from Sigma (St. Louis, MO).

For fluorescence decay measurements, samples were dissolved in 0.1 *M* pH 7.4 phosphate buffer to a level of about 1 mg/ml. Final concentrations of protein, Trp, and NATA were adjusted to give solutions having 295 nm absorbance of 0.1 units in a 1.0 cm path quartz cell. For some experiments, the protein was dissolved in 5 *M* guanidine hydrochloride solution in 0.1 *M* phosphate buffer also at pH 7.4. The lifetime measurements were done with samples in 1.0 cm path length quartz cells containing 1 ml of solution.

Steady state absorption and fluorescence emission spectra of the crystallin and reference solutions were routinely checked using a Shimadzu UV-3100 spectrometer and a Spex fluorolog-2 fluorimeter, respectively. All absorption and emission spectra were comparable to previous data from the literature [6,7] and are not reproduced here. The quantum yield of γ-II was determined relative to that of NATA by preparing solutions having equal absorbance of 0.1 at 295 nm and then comparing the areas under their corrected fluorescence emission curves excited at 295 nm. Under these conditions, absorption and emission by tyrosine residues in γ-II crystallin are negligible relative to that of the tryptophan residues.

To determine the possible effect of dissolved oxygen (O_2) on the measured fluorescence decays, some solutions were bubbled with oxygen or nitrogen gas for 30 minutes immediately prior to fluorescence lifetime measurements.

The apparatus for picosecond lifetime measurements has been described elsewhere [32]. A Coherent Antares 76-S mode locked YAG laser pumped rhodamine-6G in a Spectra Physics 375-00 dye laser with Spectra Physics 344 cavity dumper to produce a 3.8 MHz train of 10 ps pulses at 590 nm. The output of the dye laser was doubled with an angle tuned KTP crystal to produce radiation at 295 rim. The 590 nm light was removed from the excitation beam using a cutoff filter. The intrinsic polarization of the 295 nm beam was horizontal, and a half-wave plate was used to rotate the polarization to the vertical.

This beam was used to excite the samples. The instrument response function was determined by measuring the intensity profile of 295 nm radiation scattered from phosphate buffer solution containing a drop of milk. The full width at half maximum of this profile was typically 80 ps, as seen in Fig. 1.

Fluorescence decay was monitored at a 90° angle to the excitation beam at discrete emission wavelengths in the range 320–420 nm using a Nikon P250 monochromator between the sample and the Hamamatsu channel plate photomultiplier. A 310 nm cut-off filter was interposed between the sample and emission monochromator to remove any residual scattered exciting light. A Glan prism polarizer set at the magic angle of 54.7° was also placed between the sample and monochromator.

Photon counting rates were kept below 10,000 cps, and data were collected to yield 1000–10,000 counts in the maximum channel. This typically required 5–20 minutes per sample depending on the emission quantum yield. All measurements were done at room temperature of 23 ± 1°C except as noted. For temperature variation studies, a thermostated water–ethanol bath was circulated through a brass jacket surrounding the sample. Temperatures were stable to within ± 0.2°C. At least three fluorescence decays were recorded under each set of experimental conditions, and numerical data presented are averages.

Fluorescence decay data were analyzed using the GLOBAL software package [33]. Decay functions containing one, two, or three exponential components were convoluted with the instrument response function and fitted to the experimental decay data. The quality of the fits was characterized in terms of the reduced chi-squared value, the distribution of residuals, and the autocorrelation function of the residuals. With these procedures, decay components as short as 20 ps could be recovered, but no decays shorter than 40 ps are reported in the present work. Although the GLOBAL software is capable of treating complex fluorescence decay situations involving excited state reactions and energy transfer, we were able to fit all of our fluorescence decay data as sums of single, double and triple exponentials.

3. Results

A typical decay trace, together with instrument response function and the autocorrelation function of the residuals to the least-squares fit, for γ-II crystallin in pH 7.4 phosphate buffer excited at 295 nm and detected at 320 nm and 23°C is shown in Fig. 1. This provides a graphic example of the typical quality of data and the fit using the GLOBAL least squares routine. The lifetimes for a two component fit to the data in Fig. 1 yielded: $\tau_1 = 1.3$ ns and $\tau_2 = 220$ ps with chi-squared of 1.4. A three-exponent model gave: $\tau_1 = 1.5$ ns, $\tau_2 = 430$ ps, and $\tau_3 = 40$ ps with a chi-squared of 1.03. A single exponential fit was wholly inadequate as is clearly evident by the nonlinear nature of the decay in Fig. 1.

The γ-II crystallin protein showed no evidence for a rising component in the decay data (as would be indicated by a negative pre-exponential weighting coefficient α_i among the fitting parameters). The decay for the reference *N*-acetyltryptophan amide (NATA) in phosphate buffer solution detected at 360 nm yielded a linear plot as shown in Fig. 2. This data was fit to a single exponential decay model with a lifetime of 2.9 ns and chi-squared of 1.00, in agreement with the

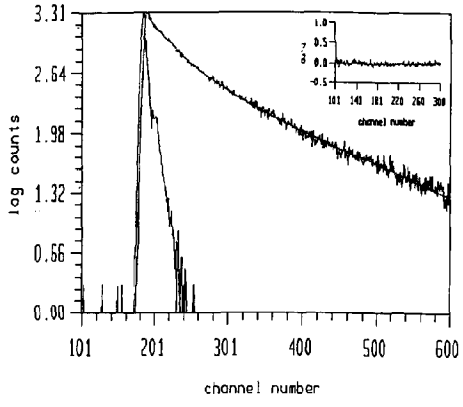

Fig. 1. Fluorescence decay data at 320 nm for bovine γ-II crystallin in pH 7.4 Buffer solution at 23°C excited at 295 nm. The instrument response function is also shown. Analysis yielded lifetime components of 1.3 ns (30%) and 220 ps (70%) with a chi-squared value of 1.4. The inset shows the autocorrelation function of the least-squares residuals. The time scale is 12.2 ps/ch.

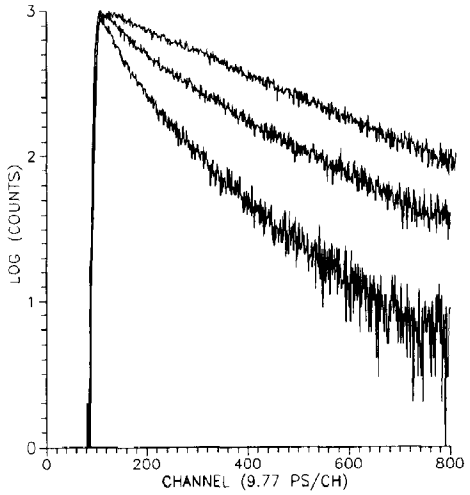

Fig. 2. Fluorescence decay curves in pH 7.4 phosphate buffer solutions at 23°C. Top to bottom: NATA (360 nm detection), γ-II crystallin (340 nm detection) in 5 *M* guanidine hydrochloride, γ-II crystallin (340 nm detection) in buffer. Excitation is at 295 nm.

previous report of Petrich et al., [34]. The observed mono-exponential decay in NATA helped to establish that the complex decay and short lifetime components observed in γ-II crystallin were not the result of impurities in the solvent, or artifacts arising from the cuvette or optics. Complete numerical parameters for γ-II crystallin and for reference materials tryptophan and NATA are shown in Table 1 for 295 nm excitation and several emission wavelengths.

The decay of γ-II crystallin in buffer solution was adequately fit by a two-exponent decay model in most data sets ($\chi^2 = 1.1$–1.5 range), but some decays were better fit by a three-exponential model which reduced χ^2 to the range 0.9–1.1. Typical results of the two-exponent fit are given in Table 1 as a function of monitored emission wavelength. When fit as a three-exponential decay, γ-II crystallin yielded a very short component τ_3 of order 40 ps in addition to the nanosecond and hundred-picosecond components recovered in the two-exponential model. Although there may be *bona fide* examples of protein fluorescence decay components as short as 40–70 ps in the literature [35,36], other authors have attributed such very short decays to Rayleigh or Raman scattered light [37]. Since a three-exponent model was only marginally justified by our data, we have not pursued this more complex analysis and present only results from the biexponential decay model.

The decay data for γ-II crystallin in Table 1 showed increasing lifetime as a function of detection wavelength. In Table 2 we indicate the degree of dependence of the fluorescence lifetime on monitored wavelength for both the long and short decays τ_1 and τ_2. In Table 2 the widths $\Delta\tau_1$ and $\Delta\tau_2$ express the variation in lifetime over the range of detection wavelengths from 320–400 nm

Table 1

Fluorescence decay parameters for γ-II crystallin protein at 23°C [a]

Sample	Detection wavelength (nm) [b,c]									
	320		340		360		380		400	
	τ_1 (α_1) (ns)	τ_2 (ns)	τ_1 (α_1) (ns)	τ_2 (ns)	τ_1 (α_1) (ns)	τ_2 (ns)	τ_1 (α_1) (ns)	τ_2 (ns)	τ_1 (α_1) (ns)	τ_2 (ns)
Gamma-II	1.3 (0.3)	0.22	1.5 (0.3)	0.25	1.8 (0.3)	0.30	2.3 (0.3)	0.32	2.6 (0.4)	0.33
Gamma-II/Gu [d]	2.4 (0.6)	0.41	2.4 (0.6)	0.45	2.5 (0.6)	0.50	2.5 (0.6)	0.48	2.6 (0.6)	0.46
NATA	2.7 (1.0)	–	2.8 (1.0)	–	2.9 (1.0)	–	2.9 (1.0)	–	3.0 (1.0)	–
Tryptophan	2.7 (0.5)	0.48	2.8 (0.7)	0.55	2.8 (0.8)	0.60	2.9 (0.9)	0.62	3.1 (0.9)	0.65

[a] All samples in pH 7.4 phosphate buffer unless otherwise noted. The excitation wavelength was 295 nm.

[b] For each detection wavelength the longer decay component is τ_1. The α_1 value in parenthesis is the weighting factor for τ_1. For two components, $\alpha_1 + \alpha_2 = 1.0$, and thus α_2 is not shown.

[c] All lifetimes are in nanoseconds and are averages of at least three measurements. Estimated uncertainties are ±0.1 ns.

[d] Guanidine hydrochloride (5 *M*) solvent.

Table 2

Distribution of fluorescence lifetimes versus wavelength [a,b]

Sample	τ_1 (ns)	$\delta\tau_1$ (%)	τ_2 (ns)	$\Delta\tau_2$ (%)
γ-II	1.8	72	0.30	37
γ-II/G-HCl [c]	2.5	8	0.50	10
NATA [d]	2.9	10	–	–
Tryptophan	2.8	14	0.60	28

[a] All data for pH 7.4 phosphate buffer at 23°C. Lifetimes in columns labelled τ_1 and τ_2 were detected at 360 nm and are expressed in nanosecond units. Most decays were double exponentials with two lifetime components τ_1 and τ_2. The excitation wavelength was 295 nm in all cases.

[b] The data given in the columns labelled $\Delta\tau$ represent the range of measured lifetimes for fluorescence detection at emission wavelengths of 320–400 nm (see Table 1), divided by the lifetime value at 360 nm.

[c] Guanidine hydrochloride solution, 5 *M*.

[d] Monoexponential decay.

expressed as a percent of the value at 360 nm. Native γ-II crystallin is seen to have the greatest inhomogeneity in its long lifetime component (72%), while its short component varied much less with detection wavelength (37%). The greatest homogeneity was found in NATA (10% variation in its single decay) and in γ-II crystallin in guanidine HCl, with 8% variation in the long decay component and 10% variation in the short decay component. Tryptophan was comparable but showed slightly greater inhomogeneity than the preceeding two examples.

The effect of the denaturing solvent 5 *M* guanidine hydrochloride on the fluorescence decay of γ-II crystallin excited at 295 nm and detected at 340 nm and 23°C is shown in Fig. 2. The decay in buffer solution was fit to a two component decay with $\tau_1 = 1.5$ ns (30%) and $\tau_2 = 250$ ps (70%). The decay rate in guanidine hydrochloride was significantly reduced relative to buffer solution, but the decay in guanidine hydrochloride still required a two-component fit, yielding $\tau_1 = 2.4$ ns (60%) and $\tau_2 = 450$ ps (40%). Thus, both the long and short lived decays increased upon protein denaturation, and the relative intensity of the long component increased at the expense of the short component. The data in Fig. 2, including the monoexponential decay of NATA for comparison, shows that denaturation of γ-II crystallin in guanidine hydrochloride caused the fluorescence decay to approach more closely to the free fluorophore limit exemplified by NATA, but that the transition was not complete. The fluorescence decays measured after one hour and twenty hours in guanidine hydrochloride were the same. Similar results were obtained at other detection wavelengths as shown in Table 1. It is noteworthy that the decay parameters for γ-II in guanidine hydrochloride were almost completely independent of detection wavelength as seen in Tables 1 and 2. This was definitely not the case for γ-II crystallin in buffer solution.

The fluorescence quantum yield of γ-II crystallin in pH 7.4 phosphate buffer solution was

Table 3

Temperature dependence of fluorescence lifetimes in NATA and γ-II crystallin [a]

Temp. (°C)	NATA [b] wavelength (nm)			Gamma-II [c] wavelength (nm)					
	330	350	380	330		350		380	
0	3.6	3.7	3.9	1.7	0.28	1.9	0.27	2.8	0.42
10	–	–	–	1.6	0.29	1.8	0.29	2.7	0.39
20	2.8	2.9	3.1	1.5	0.25	1.7	0.28	2.5	0.35
30	–	–	–	1.4	0.22	1.7	0.28	2.5	0.31
40	1.8	1.9	2.0	1.4	0.20	1.6	0.25	2.2	0.25
50	–	–	–	1.3	0.19	1.5	0.21	2.1	0.24

[a] All values in nanoseconds. Decays measured in aqueous phosphate buffer solutions at pH 7.4. Excitation at 295 nm.

[b] Decays for NATA were monoexponential at all temperatures.

[c] Decays for γ-II Crystallin were all double exponentials and hence two values are given for each wavelength and temperature.

determined (relative to NATA with $\phi_F = 0.14$, [38]) to be $\phi_F = 0.040 \pm 0.005$. This value was in good agreement with the value of 0.043 reported by Mandal et al. [6], after correcting their tryptophan reference value to $\phi_F = 0.14$ of Robbins et al. [38], rather than the value of 0.20 which they used. Since, the radiative rate constant k_R is

$$k_R = \phi_F / \langle \tau \rangle \quad (1)$$

where $\langle \tau \rangle$ is the average lifetime, defined by

$$\langle \tau \rangle = \Sigma_i \alpha_i \tau_i \quad (2)$$

we calculated $k_R = (5.3 \pm 1.0) \times 10^7\ s^{-1}$ using α_i and τ_i values from Table 1. This number is equal within experimental error to the value $(5.0 \pm 0.5) \times 10^7\ s^{-1}$, which we obtained for NATA, and with values in the literature ranging from 4.5×10^7 to $6.3 \times 10^7 s^{-1}$ for various indole derivatives [34,39]. We also measured the fluorescence quantum yield of γ-II crystallin in guanidine hydrochloride solution and found $\phi_F = 0.069 \pm 0.01$. Calculating $\langle \tau \rangle$ from eq. (2) and data in Table 1, eq. (1) yields $k_R = (4.7 \pm 1.0) \times 10^7\ s^{-1}$ again in agreement with the value for NATA [34].

In order to determine if the rather fast decay observed for γ-II crystallin in buffer solution (Table 1) was the result of some thermally activated process, we measured the temperature dependence of the fluorescence decay. Similar measurements were performed on NATA in buffer solution for comparison. The data are shown in Table 3. The fluorescence decays in γ-II crystallin were adequately fit as double exponentials at all temperatures in the range 0–50°C. Following the analysis of Petrich et al. [34], we defined the non-radiative rate constant k_{NR} by

$$k_{NR} = \tau^{-1} - k_R \quad (3)$$

where τ is the experimental fluorescence lifetime and $k_R = 5.0 \times 10^7\ s^{-1}$ is the radiative rate constant, assumed to be independent of temperature. Defined as in eq. (3), k_{NR} includes the singlet-to-triplet intersystem crossing rate constant which has a value of $k_{ISC} = 3.3 \times 10^7\ s^{-1}$ at 25°C [38]. Using τ values from Table 3, we calculated k_{NR} for NATA and γ-II crystallin from eq. (3) at various temperatures and plotted $\ln(k_{NR})$ vs. $1/T$ as shown in Fig. 3. Data are given for NATA and γ-II for emission wavelengths of 330, 350 and 380 nm. For γ-II, the long- and short-lived decay components were plotted separately. The slopes of these lines, as seen in Fig. 3, appeared to be independent of emission wavelength within experimental error, and the average activation energies obtained for the nonradiative decay rate constants in NATA and γ-II were: $E_a = 4.2 \pm 1$ kcal/mol for NATA, $E_a = 1.2 \pm 0.2$ kcal/mol (τ_1 component) and $E_a = 2.0 \pm 0.5$ kcal/mol (τ_2 component) for γ-II crystallin. The value for NATA is in reasonable agreement with the number 5.6 ± 0.2 kcal/mol reported by Petrich et al. [34].

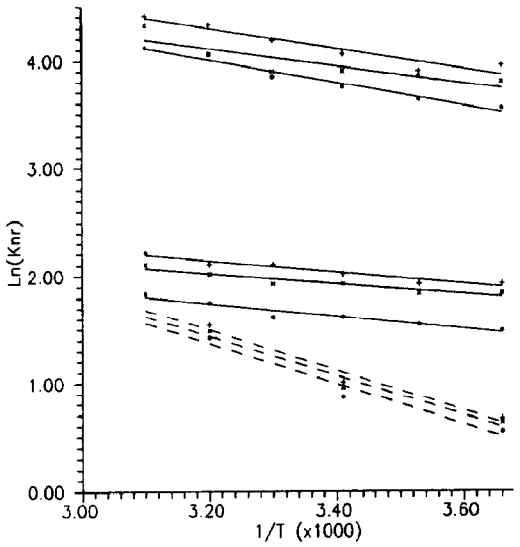

Fig. 3. Temperature dependence of non-radiative rate constants phosphate Buffer solutions at pH 7.4. Data are for NATA (— — —) and γ-II crystallin (———) for detection wavelengths of 330 nm (+), 350 nm (×) and 380 nm (*). NATA was monoexponential. Gamma-II was biexponential and data for each lifetime component are plotted separately.

The effect of dissolved molecular oxygen (O_2) was investigated by bubbling oxygen or nitrogen gas through γ-II crystallin buffer solutions for 30 min prior to making lifetime measurements. Similar measurements were performed for tryptophan monomer in buffer solution as a reference. Nei-

ther O_2(g) nor N_2(g) bubbling had any measurable effect on the fluorescence decay of γ-II crystallin or on tryptophan monomer.

4. Discussion

According to one simple model [40], the degree of lifetime shortening in a protein (relative to a hydrophilic standard like tryptophan monomer or NATA) is a measure of the average degree of hydrophobicity of the emitting tryptophan residue(s) in the protein. On this basis, our data suggests that the Trp residues in γ-II crystallin exist in a highly hydrophobic environment. This is reasonable based on previous fluorescence spectral data and fluorescence quenching data [41].

The γ-II crystallin showed a pronounced increase in decay times as a function of monitored emission wavelength (Tables 1 and 2). This phenomenon is well known [36] and may be taken as a measure of inhomogeneity of the environment of the emitting tryptophan residues. The $\Delta\tau$ values in Table 2 indicate that native γ-II crystallin possesses a much higher degree of emitter inhomogeneity than the standards or the denatured protein.

Our fluorescence quantum yield and lifetime data for γ-II crystallin show that the radiative rate constant is equal to that observed in other indole derivatives [34,39], and hence the short lifetimes in γ-II are due to efficient radiationless relaxation of the emitting tryptophan residues.

The relatively short fluorescence decay times recovered for γ-II crystallin in the present work are indicative of tryptophan residues in hydrophobic environments, and are thus consistent with previous fluorescence emission data [6], acrylamide quenching results [41], and the X-ray structure [18], which showed the four tryptophan residues of γ-II crystallin to be buried in the hydrophobic core of the protein. The lifetime of the longer component, τ_1, is quite short compared to many proteins, and the short-lived component, τ_2, is also relatively low compared to other proteins reviewed by Beechem and Brand [1]. This lifetime shortening could result from interactions between the indole rings of tryptophan and sulfur atoms at various sites [35]. The X-ray structure of γ-II crystallin shows that the protein is very rich in Cys, His, and Met residues. Trp-42 is in contact with Cys-74, Trp-64 is in contact with Cys-32, and, and Met-86 is in contact with Trp-125 [18].

In guanidine hydrochloride solution the lifetime components of γ-II crystallin were significantly lengthened relative to buffer solution. This suggests that at least some of the lifetime shortening in the native protein is conformation dependent. The fact that double exponential decay, with shortened lifetime components, was observed even in guanidine hydrochloride solution is consistent with a similar report by Grinvald and Steinberg [42] for several other proteins and the report that γ-II crystallin is particularly resistant to both chemical and thermal denaturation [43]. It is possible that some residual tertiary structure remains even in guanidine hydrochloride solution. An alternative possibility is that some of the lifetime shortening results from tryptophan interactions with neighboring groups in the primary structure of the protein, although this would not be the case for the sulfur atom interactions noted above since these do not involve sequence neighbor residues. The fact that the fluorescence decay in guanidine hydrochloride seems to be largely independent of monitoring wavelength suggests that the Trp residues in the denatured protein exist in homogeneous environments but that this environment is substantially different from that experienced by the indole ring in the reference compound NATA.

The activation energy which we observed for non-radiative relaxation in γ-II crystallin was smaller than that of NATA. The NATA activation energy has been attributed to a rotation barrier about the C=C bond according to the "rotamer model" [34]. Our data suggest that the significant lifetime shortening in γ-II crystallin stems from some interaction not present in the monomeric model NATA. This inactivation mechanism, however, must not require a major conformational charge involving an energy barrier greater than the 1–2 kcal/mol which we observed. This would be consistent with the close

proximity of several of the indole rings to sulfur atoms in the three-dimensional structure of the protein [18].

The idea of assigning the decay components of γ-II crystallin to individual tryptophan residues is an attractive one. For example, it is known that Trp-42 undergoes particularly rapid photochemical alteration upon exposure of the native protein to UV radiation [44,45]. This photochemical reaction could provide a fast deactivation channel for the excited singlet state of tryptophan and hence be responsible for the observed short-lived decay component of $\tau_2 = 250$ ps. However, studies of other proteins in which the individual tryptophan residues have been replaced by point mutations demonstrate the pitfalls of such assignments [37]. In addition, tryptophan monomer in solution is known to display double-exponential decay kinetics [34]. Since γ-II crystallin has four tryptophans, there are, in principle, eight or more possible decay components present in the protein. Many of these may be impossible to resolve under typical signal to noise conditions. Thus, it is dangerous to assign the γ-II decay components to particular tryptophan residues in the absence of more detailed information than is presently available.

The possibility of Trp-to-Trp electronic energy transfer in γ-II crystallin should be considered if the Trp residues are within a distance less than or about equal to the Forster critical transfer distance, R_0. The value of R_0 for Trp-to-Trp energy transfer depends on the relative angular orientation of the indole rings and on the energy gap between their excited singlet states. Typically R_0 lies in the range of about 6–12 Å [36,46]. Examination of the X-ray structure [18] shows that none of the Trp–Trp distances in γ-II crystallin is less than 12 Å. Hence, Trp–Trp singlet energy transfer is not likely to be important.

The lack of fluorescence lifetime shortening by addition of O_2(g) at atmospheric pressure, and the failure to observe any fluorescence lifetime increase upon deoxygenation by N_2 purging, were not surprising. The fluorescence lifetimes of tryptophan and γ-II crystallin ($\lesssim 3$ ns) are too short to permit quenching by oxygen with an expected diffusion rate constant of 10^{10} M^{-1} s^{-1} and oxygen concentrations of ca. 10^{-4} M for air-saturated or ca. 5×10^{-4} M for O_2-saturated water solutions at 23°C and atmospheric pressure [47].

Conclusions

The fluorescence of the lens structural protein γ-II crystallin decays much more rapidly than typical proteins. This rapid decay results from efficient non-radiative decay of the excited singlet state of one or more of the tryptophan residues in a nearly temperature independent process. Denaturing the protein in guanidine hydrochloride slows the decay rate by about a factor of two but the decay remains biexponential and significantly faster than in the monomeric model NATA.

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