Picosecond Fluorescence Decay in Photolyzed Lens Protein α-Crystallin†

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ABSTRACT: Photolysis of calf lens protein α-crystallin in aqueous solutions has been monitored by observing changes in fluorescence decay following UV irradiation at 308 nm. The fluorescence decay was biexponential in dark controls and in photolyzed solutions. The recovered lifetime components in pH 7.4 phosphate buffer at 23 °C were 3.5 and 0.5 ns before irradiation and 2.7 and 0.5 ns after irradiation. As the UV dose increased, the relative weighting coefficient of the 2.7-ns decay component decreased, and that of the 0.5-ns component increased, resulting in an overall lifetime shortening. Similar results were obtained in 5 M guanidine hydrochloride solutions where lifetime components of 2.7 and 0.5 ns were observed. These observations were in contrast to the behavior of tryptophan monomer solutions which did not show any change in fluorescence decay kinetics upon UV photolysis but only a reduced fluorescence intensity. Steady-state fluorescence spectra and fluorescence quantum yields were also measured at 23 °C for unirradiated bovine α-crystallin and gave $\phi_F = 0.11 \pm 0.01$ in pH 7.4 buffer and $\phi_F = 0.10 \pm 0.01$ in 5 M guanidine hydrochloride solutions. The combined steady-state and fluorescence decay data were consistent with assignment of the long-lived fluorescence decay component in α-crystallin to emission from Trp-9, which is known to photolyze relatively rapidly. The short decay component was assigned to Trp-60, which photolyzed much more slowly. We thus provide an example of using steady-state photochemical data to assign fluorescence decay components in a multi-tryptophan protein.

The photochemistry of tryptophan is dependent on the hydrophobicity of its environment (Grossweiner, 1976) and on the presence of charges, other residues, and specific chemical functionalities near the indole ring (Bent & Hayon, 1975; Pernot & Lindqvist, 1976; Tassin & Borkman, 1980; Dillon, 1981; Land et al., 1983). Examples of enhanced photochemical reactivity in particular tryptophan residues have been observed in the lens proteins α-crystallin (Dillon et al., 1987; McDermott et al., 1991) and γ_{II}-crystallin (Tallmadge & Borkman, 1990; Hott & Borkman, 1992) and in various proteins and peptides in which Trp residues are near electron acceptors (Pigault & Gerard, 1989).

Photochemical modification of the lens proteins is of interest because wavelengths greater than about 300 nm, from sunlight and other ambient sources, are transmitted by the cornea and absorbed by the proteins of the lens (Bachem, 1956). It has been suggested that photochemical modification of lens proteins may be a contributing factor in human cataracts (Borkman, 1984; Pitts et al., 1986; Andley, 1987).

The photochemical reactions of tryptophan must compete in time with primary photophysical processes of indole, including fluorescence, phosphorescence, radiationless deactivation, and photoionization (Bazin et al., 1983). The decay of fluorescence from tryptophan residues in proteins and peptides is extremely sensitive to the local environments of the emitting indoles (Beechem & Brand, 1985). In general, the fluorescence decay of proteins is complex and must be represented by a sum of several exponential decay components. In some cases, it has been possible to assign particular decay components to specific tryptophan residues (Ross et al., 1981). The evidence for such assignments often derives from comparative data on site-mutated proteins in which one or more Trp residues are replaced by nonfluorescent residues (Axelsen et al., 1991; Locke et al., 1992).

The α-crystallin fraction of calf lens is an oligomer of 800 kDa. The oligomer is composed of two different polypeptide chains, A_2 and B_2, of known sequence (Van der Ouderaa et al., 1973, 1974). The A_2 chain has a molecular weight of 19 832 and one tryptophan residue, Trp-9. The B_2 chain has a molecular weight of 20 070 and two tryptophan residues, Trp-9 and Trp-60. Dillon, Chiesa, and Spector (1987) used knowledge of the tryptic peptide map of α-crystallin to show that photolysis of the protein at $\lambda > 293$ nm in phosphate buffer solutions at neutral pH resulted in up to 90% loss of Trp-9 with negligible loss of Trp-60. The direct photolysis work of Dillon et al. (1987) made no effort to distinguish between the photolysis rates of the Trp-9 residues in the A_2 and B_2 polypeptide chains of bovine α-crystallin. The work of McDermott et al. (1991), using photosensitized conditions, indicated approximately equal rates of photolysis for Trp-9 in the A_2 and B_2 polypeptide chains.

In the present paper, we observe changes in the fluorescence decay kinetics of calf lens α-crystallin following UV irradiation. The changes are interpreted in terms of altered numbers of emitting tryptophan residues due to photochemical modification of indole rings in the A_2 and B_2 peptide chains as reported (Dillon et al., 1987; McDermott et al., 1991) and in terms of possible protein structure modifications accompanying photolysis and cataract, as has been suggested previously (Andley et al., 1982; Andley & Chapman, 1986; Harding, 1972, 1981).

The only previously published fluorescence lifetime data on bovine α-crystallin are the 10-year-old results of Borkman

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et al. (1980, 1981b) which were obtained with a nanosecond time-resolution instrument. No other lifetime data on photolyzed crystallins have been reported to date.

MATERIALS AND METHODS

Calf α-crystallin was obtained from two sources: first, from Sigma, St. Louis, MO; second, according to the procedure described previously (Hott & Borkman, 1993) and briefly summarized below. Tryptophan was obtained from Sigma.

The bovine lens protein fractions α-, β_H-, β_L-, and γ-crystallin were separated using Sephadex G-200 according to Bloemendal's (1981) procedure. The proteins were eluted with a buffer containing 1% ammonium bicarbonate (Fisher Chemical Co., Atlanta, GA) and 0.01 M β-mercaptoethanol (Sigma Chemical Co.), pH 8.1. The soluble fraction of a single lens homogenate was separated on a 2.5 × 100 cm column at a flow rate of 0.5 mL/min for 30 h. All of the separations were achieved in a 4 °C cold box. After separation, the α-crystallin fraction was lyophilized and stored at −5 °C until needed.

For UV irradiation experiments, α-crystallin was dissolved in 0.1 M pH 7.4 phosphate buffer to a level of about 1 mg/mL. The final concentration was adjusted to give solutions having a 295-nm absorbance of 0.1 unit 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 UV-irradiated sample cells contained 2.0 mL of solution.

UV irradiations at 308 nm were done using XeCl as the lasing medium in a Lambda Physik Model EMG-104 excimer laser for times of 0–8 min. The laser beam was not focused and yielded an illuminated area of about 1 cm × 1 cm. The energy output was 40 mJ per 10-ns pulse, and the repetition rate was 10 Hz. This indicated incident doses of 0–200 J. However, since the absorbances at 308 nm were only a few percent, the energy absorbed in typical UV irradiation experiments was in the range 0–10 J. This corresponded to about 30–100 photons absorbed per Trp residue in our photolysis experiments and indicates a photolysis quantum yield of a few percent. We have no direct information on the relative importance of single vs multiphoton events in the photolysis reaction. Our estimated photolysis quantum yield of a few percent is, however, similar to photoionization data (Steen, 1974) and photolysis data (Pailthorpe et al., 1973) obtained for tryptophan monomer with nonlaser sources. The laser beam was not focused. Both of these points might argue against a major component of multiphoton chemistry, but we cannot rule out the possibility.

The steady-state absorption and fluorescence emission spectra of α-crystallin and tryptophan solutions were measured before and after UV exposure using a Shimadzu UV-3100 spectrometer and a Spex Fluorolog-2 fluorometer, respectively.

The apparatus for picosecond lifetime measurements has been described elsewhere (Yamazaki et al., 1985). A coherent Antares 76-S mode-locked YAG laser pumped rhodamine-6G in a Spectra Physics 375-00 dye laser with a Spectra Physics 344 cavity dumper to produce a 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 nm. The intrinsic polarization of this beam was horizontal, and a 300-nm half-wave plate was used to rotate the plane of polarization to the vertical. This beam was used to excite the samples. Fluorescence at a 90° angle to the excitation beam passed through a Glan prism polarizer set at the magic angle of 54.7° and a Nikon P-250 monochromator and was detected by a

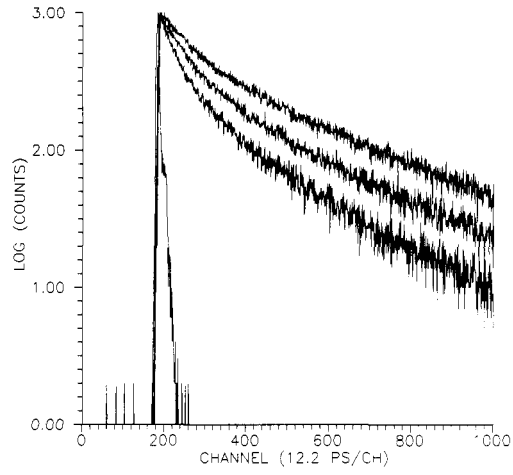

FIGURE 1: Fluorescence decay data at an emission wavelength of 360 nm for bovine α-crystallin in pH 7.4 buffer solution at 23 °C excited at 295 nm. The three curves (top to bottom) represent 0, 2, and 8 min of UV irradiation at 308 nm. The instrument response function is also shown.

Hamamatsu channel plate photomultiplier and conventional photon counting electronics. A 320-nm cutoff filter was interposed between the sample and emission monochromator to remove scattered exciting light. Fluorescence decay was monitored at discrete emission wavelengths in the range 320–420 nm. The instrument response function was determined by measuring the intensity profile of radiation at 295 nm scattered from phosphate buffer solution containing a drop of milk. Although the milk proteins could, in principle, have contributed fluorescence of their own, we observed no scatter or fluorescence beyond the background dark count rate when the detection monochromator was set at wavelenths greater than 295 nm, indicating that milk fluorescence was not important. The full width at half-maximum of the scattered radiation profile was typically 80 ps, as seen in Figures 1 and 2. Data were collected to give 1000–10 000 counts in the maximum channel which typically required 10–20 min per sample. All measurements were at 23 ± 1 °C.

Fluorescence decay data were analyzed using the Global software package (Beecham et al., 1990). Decay functions containing one-, two-, or three-exponential components were convoluted with the instrument response function and fitted to the individual experimental decays. The quality of the fits was characterized in terms of the reduced χ^2 value, the distribution of residuals, and the autocorrelation function of the residuals. With these procedures, decay components as short as 20 ps could be reliably recovered. All decays were successfully modeled as single, double, or triple exponentials. There was no evidence for nonexponential decay of the type expected when fluorescence quenching or energy transfer is involved (Beecham et al., 1990).

RESULTS

Fluorescence decay data (excitation 295 nm, emission 360 nm) for bovine α-crystallin in pH 7.4 phosphate buffer, before and after exposure to UV radiation at 308 nm for times up to 8 min, are shown in Figure 1. The instrument response function is also shown to give an indication of the time resolution of the experiment. Fluorescence decay data for α-crystallin in guanidine hydrochloride solution are shown in Figure 2. There was a very striking difference between the decay curves for α-crystallin fluorescence before and after UV irradiation, with the fluorescence decay rate in the irradiated samples being accelerated relative to the dark

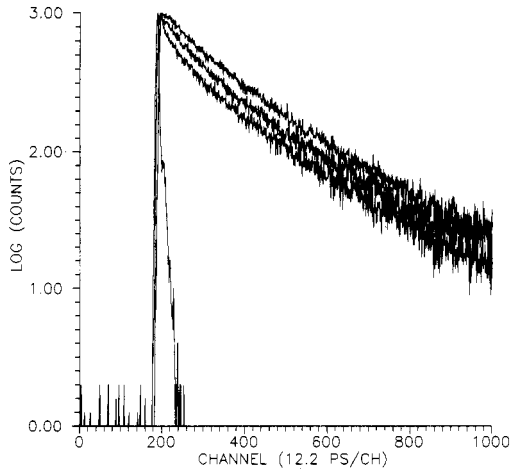

FIGURE 2: Fluorescence decay data at 360 nm for bovine α-crystallin in 5 M guanidine hydrochloride solution at 23 °C excited at 295 nm. The three curves (top to bottom) represent 0, 4, and 8 min of UV irradiation at 308 nm. The instrument response function is also shown.

Table I: Fluorescence Decay Parameters for Lens Protein α-Crystallin in Buffer Solution at pH 7.4[a]

lifetime[b]/coefficient ratio	UV irradiation time (min)[c]			
	0	2	4	8
	340-nm Detection			
τ_1	3.2	2.7	2.7	2.7
τ_2	0.50	0.50	0.50	0.50
α_1/α_2	1.22	0.82	0.56	0.43
χ^2	1.0	1.0	1.1	1.4
	360-nm Detection			
τ_1	3.5	2.7	2.7	2.7
τ_2	0.50	0.50	0.50	0.50
α_1/α_2	1.22	1.00	0.82	0.50
χ^2	1.3	1.1	1.1	1.1

[a] Solutions of bovine α-crystallin protein in 0.1 M phosphate buffer at pH 7.4. The excitation wavelength was 295 nm in all experiments, and the detection wavelength was either 340 nm or 360 nm as shown.[b] All lifetimes are in nanoseconds and are averages of at least three determinations. Estimated uncertainties in lifetimes are ±0.1 ns. In addition to the τ_1 and τ_2 values shown, a short component, τ_3 = 20 ps, was included in each decay as discussed in the text. Typical χ^2 quality-of-fit parameters are shown for each data set. [c] Irradiation at 308 nm as described in the text.

controls. Similar behavior was seen for α-crystallin in guanidine hydrochloride solutions. In contrast, solutions of tryptophan monomer before and after UV irradiation at 308 nm gave decay traces which were indistinguishable, except that the intensity in the UV-irradiated samples was lower, presumably due to the reduced level of indole fluorophores in the photolyzed samples.

The lifetime components and weighting factors resulting from analysis of the fluorescence decay data in buffer solution at pH 7.4 are shown in Table I. Prior to UV irradiation, the fluorescence decay of α-crystallin in buffer solution, detected at 360 nm, was fit by a double-exponential model with lifetimes of τ_1 = 3.5 ns (55%) and τ_2 = 500 ps (45%). An improved fit could be obtained in many cases by adding a third, very fast exponential decay component of order τ_3 = 20 ps. [The need for such a short component in the analysis of protein fluorescence decay was reported and discussed previously by Axelsen et al. (1991).] After 2 min of UV irradiation, the best-fit decay components for α-crystallin in buffer solution were τ_1 = 2.7 ns and τ_2 = 500 ps. Similar results were obtained for other UV irradiation times and detection wavelengths as seen in Table I. It was possible to fit the individual decay data for all of the UV-irradiated samples using lifetime components of τ_1 = 2.7 ns and τ_2 = 500 ps and varying only the weighting factors α_1 and α_2. As the UV dose increased from 2 to 4 to 8 min, the coefficient ratio α_1/α_2 decreased as seen in Table I. In contrast, the decay components of tryptophan monomer fluorescence in buffer solution were essentially unaffected by similar doses of UV radiation. Before UV irradiation, we obtained τ_1 = 2.85 ns and τ_2 = 580 ps, similar to the results of Petrich et al. (1983). After irradiation, the best-fit decay components were τ_1 = 2.75 ns and τ_2 = 550 ps with no change in the weighting factors. Hence, within experimental error, there was no change in the shape of the fluorescence decay as a result of UV irradiation of tryptophan monomer in buffer solution. For α-crystallin in guanidine hydrochloride solutions, the fluorescence decay at 360 nm was characterized by lifetimes of τ_1 = 2.7 ns (66%) and τ_2 = 500 ps (34%). Addition of a short decay component, τ_3 = 20 ps, improved the fit, especially after UV irradiation. These same lifetime components, with changes in the weighting factors, α, also fit the decay after UV irradiation as seen in Table II. As the UV dose increased, the ratio of α_1 to α_2 decreased in a similar fashion to that seen in buffer solutions. The Global software (Beecham et al., 1990) is capable of sophisticated linked analyses as has been demonstrated by others (Axelsen et al., 1991; Beechem & Brand, 1986; Knutson et al., 1983; Ruggiero et al., 1990). The single-decay model used in our work yielded reasonable fits to the individual decays as judged by the reduced χ^2 and autocorrelation of residuals criteria.

Table II: Fluorescence Decay Parameters for Lens Protein α-Crystallin in Guanidine Hydrochloride Solution at pH 7.4[a]

lifetime[b]/coefficient ratio	UV irradiation time (min)[c]		
	0	4	8
	340-nm Detection		
τ_1	2.7	2.7	2.7
τ_2	0.50	0.50	0.50
α_1/α_2	1.78	1.00	0.82
χ^2	1.2	1.3	1.3
	360-nm Detection		
τ_1	2.7	2.7	2.7
τ_2	0.50	0.50	0.50
α_1/α_2	2.00	1.22	1.00
χ^2	1.3	1.2	1.4

[a] Solutions of bovine α-crystallin protein in 0.1 M phosphate buffer at pH 7.4 containing 5 M guanidine hydrochloride. The excitation wavelength was 295 nm in all experiments, and the detection wavelength was either 340 nm or 360 nm as shown. [b] All lifetimes are in nanoseconds and are averages of at least three determinations. Estimated uncertainties in lifetimes are ±0.1 ns. In addition to the τ_1 and τ_2 values shown, a short component, τ_3 = 20 ps, was included in each decay as discussed in the text. Typical χ^2 quality-of-fit parameters are shown for each data set. [c] Irradiation at 308 nm as described in the text.

The steady-state fluorescence spectra of α-crystallin in buffer solution in Figure 3 display emission characteristics of indole rings in a moderately hydrophobic environment with an emission maximum at 338 nm (Burstein et al., 1973; Borkman & Lerman, 1978). The fluorescence quantum yield of α-crystallin in buffer solution was estimated to be ϕ_F = 0.11 ± 0.01, by comparing the area under the emission curve in Figure 3 (at zero irradiation time) with that of tryptophan which was assumed to have a quantum yield of ϕ_F = 0.14 (Petrich et al., 1983). The fluorescence spectrum of α-crystallin in 5 M guanidine hydrochloride solution was also measured. This resulted in a fluorescence maximum at 345 nm and a quantum yield of ϕ_F = 0.10 ± 0.01.

An equation given by Hazen et al. (1976) can be used to calculate the number of emitting tryptophan residues in a

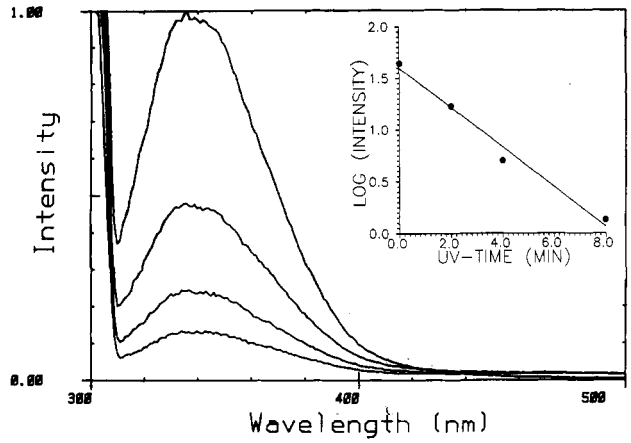

FIGURE 3: Steady-state fluorescence spectra for bovine α-crystallin in pH 7.4 aqueous buffer solution at 23 °C excited at 295 nm. The four curves (top to bottom) represent 0, 2, 4, and 8 min of UV irradiation time at 308 nm. The tail of the 295-nm excitation band can be seen in the 300–310-nm region. INSET: First-order kinetic plot of fluorescence intensity vs UV irradiation time. Intensity data are based on the areas under the emission bands in the main figure.

protein from knowledge of the fluorescence lifetime and quantum yield relative to an appropriate indole standard. Thus, the number, F, of emitting Trp residues is given by

$$F = N(\tau_0\phi_F/\langle\tau\rangle) \quad (1)$$

where N is the number of tryptophan residues per protein molecule and $\langle\tau\rangle$ is the average fluorescence lifetime of the protein:

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

τ_0 is the radiative lifetime of tryptophan and is assumed to be constant for all tryptophans in the protein and in the reference standard, and ϕ_F is the experimental fluorescence quantum yield of the protein. We used weighting coefficients, α_i, and lifetime components, τ_i, from Table I and eq 2 to calculate $\langle\tau\rangle$ = 2.16 ns at 360 nm. This, together with ϕ_F = 0.11 for α-crystallin and $\tau_0 = \tau/\phi_F$ = 21 ns for Trp monomer in eq 1, gave $F = 1.07N$. Essentially identical results were obtained using decay data at 340 nm in Table I. The resulting numbers suggest that in the native conformation of α-crystallin all Trp residues are active emitters. Repeating the calculation with data from Table II for α-crystallin in guanidine hydrochloride solution at 360 nm yielded $\langle\tau\rangle$ = 1.98 ns. This together with the fluorescence quantum yield in guanidine hydrochloride solution of ϕ_F = 0.10 and τ_0 = 21 ns gave $F = 1.06N$. This value suggests that all Trp residues in α-crystallin were fluorescent when the protein was denatured in guanidine hydrochloride. Hence, there did not seem to be evidence for strong static quenching of any of the Trp residues in either the native or the denatured forms of bovine α-crystallin.

The fluorescence intensity of α-crystallin decreased as a function of UV irradiation time, as can clearly be seen in Figure 3, presumably due to photochemical destruction of Trp residues in the protein (Borkman et al., 1981a; Fujimori, 1982; Dillon et al., 1987; Andley & Clark, 1989). (Similar results were obtained in guanidine hydrochloride solutions, but the data are not shown.) The areas under the emission curves in Figure 3, excluding the scattered light peaks in the 300–310-nm region, were measured and yielded residue losses of 34% (2 min UV), 64% (4 min UV), and 79% (8 min UV). A first-order kinetic plot of these data is shown in the inset in Figure 3. The analysis is only approximate since photolysis can also change protein conformation which can in turn affect

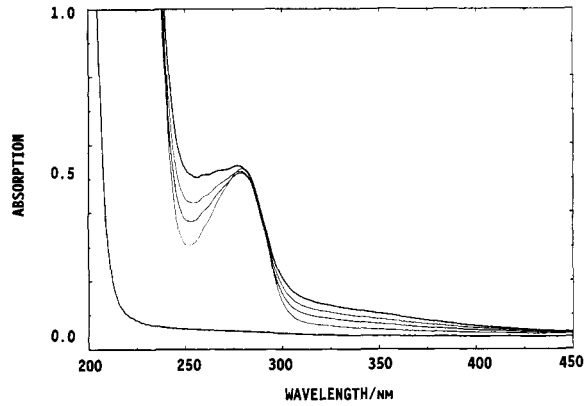

FIGURE 4: Absorption spectra for bovine α-crystallin in pH 7.4 aqueous buffer solution at 23 °C. The four curves (from bottom to top at 325 nm or at 250 nm) represent irradiation times of 0, 2, 4, and 8 min at 308 nm. The cell-plus-solvent absorption curve is also shown.

the fluorescence quantum yield. Nonetheless, the data qualitatively confirm the photolytic loss of tryptophan in α-crystallin solutions as reported previously (Dillon et al., 1987). Concomitant with the loss of Trp fluorescence in Figure 3, there was also growth of a longer wavelength fluorescence with an excitation maximum at about 340 nm and an emission maximum near 460 nm. We detected this emission but have not reproduced the spectra here since similar results have been discussed in detail elsewhere (Fujimori, 1982; Borkman et al., 1981a).

The absorption spectra of photolyzed α-crystallin solutions in Figure 4 show increasing absorption at wavelengths of about 250 nm and also in the range 300–450 nm, presumably due to increasing concentration of photoproduct(s). We did not attribute the increased absorbance to solution turbidity since the absorbance at wavelengths greater than about 450 nm (data not shown) was identical for UV-irradiated and dark control samples. In addition, the UV-irradiated α-crystallin samples did not develop any visible opacity. This result differs from our previously published observations on UV-irradiated β- and γ-crystallin solutions which showed marked absorbance increases out to 650 nm, solution opacity, and precipitation of protein (Hott & Borkman, 1993).

DISCUSSION

The most interesting result of these experiments was the striking decrease in the characteristic fluorescence decay time of α-crystallin solutions after UV irradiation. This was in contrast to our observations on photolyzed tryptophan monomer in solution which showed only a loss of fluorescence intensity but no change in the fluorescence decay kinetics. This suggests that the fluorescence decay behavior of pre-photolyzed α-crystallin solutions was dependent on the local environments of the Trp residues in the protein. There are several possible ways to attempt to explain our observations: (1) Fluorescence decay in photolyzed α-crystallin samples represented the sum of the original tryptophan emission plus a contribution from emission from one or more photoproducts. (2) Photolysis products were generated which quenched tryptophan fluorescence, hence reducing its lifetime. (3) There were local and/or global changes in protein conformation, following photolysis, which resulted in different environments and hence different lifetimes for the emitting tryptophan fluorophores. (4) The two tryptophans in α-crystallin (Trp-9 and Trp-60) have different fluorescence decay rates, and the slow-decaying Trp photolyzed more rapidly than the other, resulting in an overall decrease in the decay rate.

The possibility that photoproduct emission made a significant contribution to the total fluorescence decay of irradiated α-crystallin was not supported by the steady-state fluorescence data in Figure 3. The emission spectra of α-crystallin in the 350-nm region all appeared to be assignable to tryptophan fluorescence, at least for UV irradiation times in the range 0–8 min. Of course, a small contribution from a photoproduct having an emission similar to that of tryptophan could not be ruled out solely by the appearance of the broad, featureless spectra in Figure 3. As stated above, we observed photoproduct fluorescence in the 450-nm region, but since we used a monochromator in the fluorescence decay experiments, this longer wavelength emission did not contribute to the decay data. An additional point comes from comparing the fluorescence decay of photolyzed α-crystallin with that of photolyzed tryptophan monomer. In the latter case, although several photoproducts are known to accumulate (Borkman et al., 1986), there was no evidence in either the steady-state or the time-resolved emission to suggest a significant contribution from photoproduct fluorescence in the 350-nm region. In particular, there was no lifetime shortening in photolyzed tryptophan monomer. Finally, it can be argued that a putative fluorescent photoproduct of α-crystallin not only would have to have the same emission spectrum as the original indole emission but also would have to have the same 500-ps fluorescence decay rate as the short component of α-crystallin, since no new lifetime component was seen in the decay analysis of the photolyzed protein in Tables I and II.

The possibility that some photoproduct may have served as a fluorescence quencher of the tryptophans in α-crystallin must be carefully considered. Such quenching could have occurred at relatively long range via a Forster energy-transfer mechanism, or it could have occurred at short range via a static quenching mechanism. Either mechanism could produce lifetime shortening. However, the kinetics of the quenching process would be different. Forster energy transfer is known to produce nonexponential fluorescence decay (Beecham et al., 1990), and this was not observed in our samples of UV-irradiated α-crystallin solutions. Additionally, the kinetics of the α-crystallin photolysis reaction, depicted in Figure 3, indicated simple first-order loss of Trp fluorescence as a function of UV irradiation time. First-order kinetics would not be expected if the reaction were producing a photoproduct which was an efficient quencher of tryptophan fluorescence. Finally, a quenching mechanism would require spatial proximity between the quenched Trp residue and the photoproduct-quencher. We observed the same decay parameters for α-crystallin photolyzed in buffer and in denaturing guanidine hydrochloride solutions where many spacial arrangements are altered relative to the native form of the protein. This argues against a quenching mechanism but cannot entirely rule it out since quenching could still occur in the denatured protein if the quencher occupied a primary sequence site near that of the quenched Trp residue.

The possibility exists that some degree of local and/or global protein denaturation accompanied α-crystallin photolysis. The fluorescence decay parameters for α-crystallin in buffer solution in Table I, and in guanidine hydrochloride solution in Table II, supported this. In buffer solution, prior to irradiation, the fluorescence decay was fit by lifetime components of 3.5 and 0.5 ns. After 2-min irradiation, the long component was reduced to 2.7 ns and remained at this value for further irradiation times up to 8 min. This suggested that an early effect of protein photolysis might be a conformational change which affected the long-lived decay component and reduced its lifetime from 3.5 to 2.7 ns. This is supported by the data in Table II for guanidine hydrochloride solutions where we see that in this denaturing medium the long-lived component had a value of 2.7 ns, even before irradiation. In this case, the guanidine hydrochloride solvent may have altered the Trp environment in a similar fashion to UV irradiation.

Dillon et al. (1987) photolyzed bovine α-crystallin in buffer solutions using UV wavelengths greater than 293 nm and analyzed the resulting solutions by tryptic digestion and TLC. They found preferential destruction of Trp-9 relative to Trp-60 such that Trp-9 was 90% destroyed after 16-h irradiation with a mercury arc lamp while "there was almost no change" in the tryptic peptide containing Trp-60. These observations could account for our own results if Trp-9 were to be associated with the long-lived fluorescence decay component (3.5/2.7 ns) and Trp-60 with the short-lived decay component (0.5 ns). Photolysis would then cause a loss of the long-lived decay component and a decrease in the weighting coefficient ratio, α_1/α_2, in agreement with our experimental observations in Tables I and II.

Photolysis experiments on proteins have suggested that the degree of exposure to solvent may be an important factor in determining relative photochemical reactivities of Trp residues (Grossweiner, 1976; Pigault & Gerard, 1984). In particular, evidence supports the notion that photochemical reactivity is correlated with large solvent exposure. This means assigning the rapidly photolyzed Trp-9 in α-crystallin to a more solvent-exposed site than Trp-60. Such an assignment is consistent with the fact that the rapidly photolyzed component was the long-lived component, since Burstein et al. (1973) and Grinvald and Steinberg (1976) have associated long-lived fluorescence with solvent-exposed, hydrophilic Trp residues and short-lived fluorescence with hydrophobic Trp residues.

In summary, our data on fluorescence decay in dark-control and photolyzed bovine α-crystallin solutions are best understood in terms of the following model: The 3.5-ns decay component in unirradiated α-crystallin in buffer solution stems from Trp-9 (more solvent exposed), while the 0.5-ns component arises from Trp-60 (less solvent exposed). In guanidine hydrochloride solutions, the 3.5-ns decay is reduced to 2.7 ns as a result of alteration of the local environment of Trp-9, while the lifetime of Trp-60 remains the same as in buffer, about 0.5 ns. This suggests that the relatively short lifetime of Trp-60 arises from interaction with some groups which are nearby in the primary sequence of the B_2 peptide and hence not eliminated by global denaturation. Photolysis in buffer solution initially results in a local or global conformational change which alters the environment of the Trp-9 residue, reducing its lifetime from 3.5 to 2.7 ns. Further photolysis in buffer solutions causes preferential destruction of Trp-9 (Dillon et al., 1987) with a concomitant shortening of the overall fluorescence decay time of the protein due to the increasing relative importance of fluorescence from the short-lived Trp-60 decay. Photolysis in guanidine hydrochloride solutions also results in preferential destruction of Trp-9, resulting in an overall shorter decay time. Trp-60 seems to be protected from photolysis perhaps because its excited state is rapidly quenched by some interaction (independent of the global conformation of the protein) which rapidly returns these residues to the ground electronic state before photochemistry can occur.

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