# Photoinduced Structural Changes in the Collagen/Gelatin Binding Domain of Fibronectin†

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ABSTRACT: Prolonged exposure of human plasma fibronectin (pFn) and its 40- and 21-kDa collagen/gelatin binding fragments (GBFs) to 280-nm irradiation decreased their affinity for gelatin and for TR-CB7, a fluorescently labeled CNBr fragment of the α-1 chain of type I collagen. Fluorescence polarization binding assays of TR-CB7 with pFn and the 40-kDa GBF yielded progressively higher $K_d$'s with increased time of exposure to 280-nm light at 25 °C. Binding of nonirradiated and irradiated pFn and fragments to gelatin−Sepharose correlated with the polarization data, confirming diminished gelatin binding following exposure to 280-nm light. Fluorescence spectra of intrinsic tryptophans in the 21- and 40-kDa GBFs exhibited changes indicative of photoinduced conformational changes; the maximum fluorescence wavelength red-shifted from between 340 and 350 nm to 360 nm, with concomitant increases in fluorescence intensity. Exposure of 21- and 40-kDa GBFs and pFn to 280-nm light also generated approximately two, four, and six free sulfhydryl groups per molecule, respectively. No sulfhydryl release was observed in other Trp- and disulfide-containing proteins under the same conditions. We propose that the fluorescence changes as well as the changes in affinity for gelatin or the collagen fragment result from structural changes secondary to the breakage of disulfide bonds, as a consequence of energy transfer from nearby tryptophans in one or more of the Fn type I repeats in the gelatin binding region of fibronectin.

Fibronectrin (Fn)[1] is a large extracellular glycoprotein composed of two similar polypeptide subunits of 220−250-kDa that are connected by a pair of disulfide bonds near the C-terminus (Hynes, 1990). It is found in body fluids as well as in connective tissue where it interacts with a variety of macromolecules including integrin receptors on the surfaces of numerous cell types. Structurally, Fn is a modular protein, each chain containing multiple copies of homologous repeat sequences of three different types (I, II, and III), all of which comprise independently folded domains (Litvinovich et al., 1991; Novokhatny et al., 1992). The collagen/gelatin binding site(s) are located near the amino terminus of each chain and, after limited proteolysis, are readily isolated by affinity chromatography on gelatin−Sepharose in an ~40-kDa fragment having the modular composition $I_6$-$II_1$-$II_2$-$I_7$-$I_8$-$I_9$ (Griffin et al., 1986; Litvinovich et al., 1991). Two smaller and weaker gelatin-binding fragments, 30-kDa GBF ($I_6$-$II_1$-$II_2$-$I_7$) and 21-kDa GBF ($I_8$-$I_9$) can also be obtained by further proteolysis (Ingham et al., 1989). The type I and type II modules contain ~45 and ~60 amino acids, respectively, including two disulfide bonds whose integrity is important for gelatin binding (Ingham & Brew, 1992).

Within the 40-kDa GBF, there are a total of eight Trp and 21 Tyr residues (Skorstengaard et al., 1986). Approximately, five of the Trp residues and eight of the Tyr residues are located within one or two positions of a disulfide bond. Recent NMR-derived solution structures of recombinant Fn type I and type II domains suggest that the side chains of conserved Trp and Tyr residues are enclosed within the hydrophobic core, in close proximity to disulfide bonds (Baron et al., 1990; Williams et al., 1994; Constantine et al., 1992). Short-range interactions between fluorescent aromatic residues and disulfide bonds can lead to quenching of intrinsic fluorescence (Longworth, 1971; Cowgill, 1967). Indeed, the reduction of disulfides in the 21- and 40-kDa GBFs causes large increases in the fluorescence quantum yield with concomitant loss of gelatin-binding activity (Isaacs et al., 1989). Limited oxidation of specific Met residues in the 40-kDa GBF also disrupts gelatin binding but without perturbations in the intrinsic Trp fluorescence (Miles & Smith, 1993). We now show that extended exposure of pFn and 40- and 21-kDa GBFs to 280-nm light markedly decreases the affinity of these molecules for gelatin.[2] Furthermore, emission spectra of 280-nm irradiated samples reveal concomitant time-dependent increases in fluorescent yield and spectral red shifts that are indicative of major conformational alterations. Taken together with biochemical evidence for the generation of free sulfhydryls in irradiated samples, the results suggest that photosensitized disruption of disulfide bonds, primarily in type I domains via energy transfer from nearby tryptophans, leads to unfolding and loss of binding activity.

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[1] Abbreviations: GBF, gelatin binding fragment; TR-CB7, Texas Red-labeled CNBr fragment of type I collagen; pFn, plasma fibronectin; Fn, fibronectin; GdmCl, guanidinium chloride.

[2] A preliminary report of this work has appeared (Miles & Smith, 1994).

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## MATERIALS AND METHODS

*Chemicals and Proteins.* All chemicals were reagent grade or of the highest purity available. Citrated human plasma was supplied by the Plasmapheresis Laboratory of LSU Medical Center. Porcine skin type I gelatin was obtained from Sigma. 5,5′-Dithiobis(2-nitrobenzoate) (DTNB) was purchased from Aldrich. Plasma fibronectin (Smith & Griffin, 1985), 40-kDa GBF and its subfragments (Litvinovich et al., 1991), 29- and 19-kDa fragments (Novokhatny & Ingham, 1994), 30-kDa Hep-2 (Novokhatny et al., 1992), and 14-kDa (type $III_1$) (Litvinovich et al., 1992) and 110-kDa GBF (Borsi et al., 1986) were prepared as previously described, and their concentrations were determined from 280-nm absorbance using the extinction coefficients given in those references or calculated from the amino acid composition (Edelhoch, 1967).

*Fluorescence Measurements.* Fluorescence emission spectra and fluorescence polarization binding assays (FPB) were performed on SLM Instruments spectrofluorometers Model SPF-500C or 8000C using software supplied by the manufacturer. Fluorescence spectra were recorded at 25 °C using 1-cm path length quartz cuvettes containing solutions of protein (1.0−5.0 $\mu$M) in either TBS (Tris-buffered saline, 20 mM tris(hydroxymethyl)aminomethane, 0.15 M NaCl, pH 7.4) or 100 $\mu$M phosphate buffer, pH 7.4. Excitation wavelength was 280 nm (20-nm bandpass), and emission was recorded between 300 and 500 nm with a bandpass of 5 nm. Prolonged irradiation was accomplished by placing the sample in a 1 $cm^2$ quartz cuvette in the excitation beam in the spectrofluorometer sample compartment. The SLM SPF-500C was also equipped with a polarization accessory used in binding assays and incorporates the L-format for measurements of polarization. The details of the FPB assay have been published elsewhere (Miles & Smith, 1993).

*Detection of Sulfhydryls.* Detection of free sulfhydryls was performed as described elsewhere (Ellman, 1967). Briefly, separate 2.0 $\mu$M solutions of pFn or 21- or 40-kDa GBF were prepared in 0.1 mM phosphate buffer (pH 7.4). Each solution was then transferred separately to a 1-cm quartz cuvette and exposed continuously to 280-nm light for 20 min at 25 °C. Following irradiation, 50 $\mu$L of 3 mM dithionitrobenzoate (DTNB, freshly prepared in 1.0 mM phosphate buffer, pH 7.4) was added directly to the quartz cuvette, and the absorbance at 412 nm was monitored immediately. Based on absorbance increase, the concentration of sulfhydryls was calculated from the molar absorbance of the nitrobenzoate anion [$\epsilon_{412}$ = 14150 L $mol^{-1}$ $cm^{-1}$ (Riddles et al., 1983)]. For comparison, 2.0 $\mu$M solutions of nonirradiated protein were also prepared and analyzed as described above. All absorbance values were corrected for background.

*Electrophoresis.* Samples were electrophoresed in a Pharmacia Phast system using 8−25% polyacrylamide gradient gels in the presence of SDS, with or without prior exposure to $\beta$-mercaptoethanol, staining with Coomassie blue. Nonreduced samples were treated with iodoacetamide before electrophoresis.

## RESULTS

*Fluorescence Emission Spectra.* A series of replicate fluorescence spectra of the 40- and 21-kDa GBFs recorded at 1-min intervals are shown in Figure 1, panels A and B, respectively. The samples were continuously exposed to 280 nm light in the spectrofluorometer while recording the

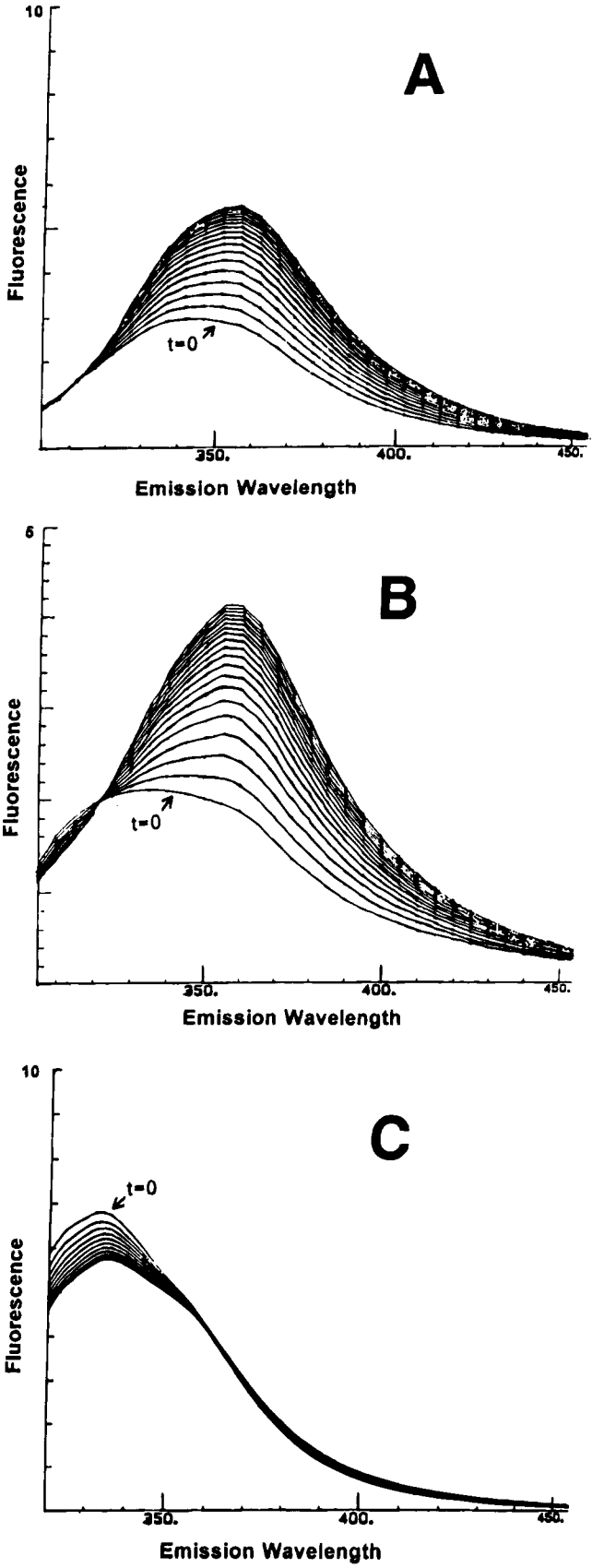


FIGURE 1: Effect of 280-nm irradiation on the fluorescence emission spectra of Fn and two of its gelatin-binding fragments. Spectra were recorded at 60-s intervals for 2.0 $\mu$M 40-kDa GBF (panel A), 2.0 $\mu$M 21-kDa GBF (panel B), and 1.0 $\mu$M pFn (panel C) in phosphate buffer, pH 7.4, at 25 °C.

Table 1: Effects of 280-nm Irradiation on Intrinsic Tryptophan Fluorescence of pFn, Its Fragments, and Other Molecules

| molecule | time of exposure (min) | increase in fluorescence (%) | $\lambda_{max}$ initial | $\lambda_{max}$ final |
|---|---|---|---|---|
| fibronectin[a] | 20 | −8 | 336 | 336 |
| 40-kDa GBF[a] ($I_6$-$II_1$-$II_2$-$I_7$-$I_8$-$I_9$) | 20 | 100 | 344 | 360 |
| 21-kDa GBF[a] ($I_8$-$I_9$) | 20 | 125 | 340 | 360 |
| 30-kDa GBF[b] ($I_6$-$II_1$-$II_2$-$I_7$) | 30 | 40 | 341 | 343 |
| 13-kDa GBF[b] ($I_6$-$II_1$) | 30 | 10 | 341 | 342 |
| 29-kDa Fib-1[b] ($I_1$-$I_2$-$I_3$-$I_4$-$I_5$) | 30 | 40 | 339 | 343 |
| 19-kDa Fib-2[b] ($I_{10}$-$I_{11}$-$I_{12}$) | 30 | 15 | 348 | 350 |
| 110-kDa CBF[b] ($III_2$–$III_{11}$) | 60 | −4 | 329 | 329 |
| 30-kDa Hep-2[b] ($III_{12}$-$III_{13}$-$III_{14}$) | 60 | −3 | 332 | 332 |
| 14-kDa Hep-3[b] ($III_1$) | 60 | −2 | 337 | 337 |
| ACTH(11-mer)[a] | 20 | ND | 360 | 360 |
| *N*-acetyl-Trp-amide[a] | 20 | ND | 360 | 360 |

[a] Performed in the SLM 500C. [b] Performed in the SLM 8000C. [c] ND, not determined.

spectra. The initial scans ($t = 0$) yielded broad spectra, with $\lambda_{max}$ near 344 nm for 40-kDa GBF and 335 nm for 21-kDa GBF. The spectra progressively red-shifted with each subsequent scan, approaching a final $\lambda_{max}$ near 360 nm for both fragments. Concomitantly, the fluorescence intensities increased in a time-dependent manner reaching values at least 2-fold higher than those which prevailed at the onset. The effect appeared to be more pronounced and occurred more rapidly with the 21-kDa fragment. A $\lambda_{max}$ of 360 nm for Trp fluorescence represents full solvent exposure of otherwise buried Trp residues as determined from the spectra of peptides and model compounds such as *N*-acetyl tryptophanamide. Two additional subfragments of 40-kDa GBF were also examined (data not shown, see Table 1). The first, 30-kDa GBF ($I_6$-$II_1$-$II_2$-$I_7$), representing the difference between the 40- and 21-kDa fragments (Ingham et al., 1989), produced an ~40% increase in the intensity after 30 min exposure to 280-nm excitation, but its $\lambda_{max}$ did not reach 360 nm even after 20-h exposure. A 13-kDa fragment ($I_6$-$II_1$) produced only a 10% increase in fluorescence after 30 min with a shift in $\lambda_{max}$ from 341 to 342 nm.

The 21-kDa fragment consists of the last two type I domains, $I_8$ and $I_9$, of 40-kDa GBF and contains seven Tyr and three Trp residues (Skorstengaard et al., 1986). The fact that its response to 280-nm radiation was greater than that of 40-kDa GBF or other subfragments, which contain type II as well as type I domains, suggested that the observed effect might be most prevalent in type I domains. Furthermore, when whole Fn was examined, it underwent a decrease rather than an increase in fluorescence intensity with only a minor change in wavelength (Figure 1C). We therefore examined several additional fragments containing exclusively type I domains. The results are summarized in Table 1. The 29-kDa N-terminal fragment, containing five type I domains, had an initial $\lambda_{max}$ near 339 nm, which shifted to 343 nm after 30 min exposure, with an 40% increase in overall intensity. The 19-kDa C terminal fragment, containing three type I domains, had a $\lambda_{max}$ near 348 nm and underwent an ~15% increase in intensity with a shift in $\lambda_{max}$ to 350 nm. Two additional fragments, a 110-kDa cell binding fragment and a 30-kDa heparin-binding fragment, containing exclusively type III domains with single Trp residues at identical positions showed only marginal decreases in their fluorescence intensities. The same was true for a 14-kDa fragment containing a single type III domain, number 1, which contains two additional Trps. Several other proteins failed to show a significant change after 1-h exposure. These results suggest that the observed effect is unique to type I but that not all type I repeats are equally responsive. The greatest effect was with 21-kDa GBF-containing type I domains 8 and 9. Allowing the 40- and 21-kDa fragments to stand in the dark either on ice or at 25 °C for up to 16 h after irradiation did not reverse the light-induced spectral changes, indicating that the structural changes are essentially irreversible. Irradiation of the 21-kDa fragment at 295 nm, where only Trp absorbs, produced fluorescence changes comparable to those observed with irradiation at 280 nm, indicating that excitation of tyrosine(s) is not required for the effect.

The spectral changes previously identified in connection with GBF and other type I containing fragments were not evident in whole Fn. We suspect that these may have been masked by the fluorescent events involving Trp residues located in other regions of the molecule, which showed a decrease in fluorescence upon irradiation. That changes in the gelatin binding region of whole Fn did occur was shown by measurements of the gelatin binding function.

*Light-Induced Changes in Binding Affinity.* To determine whether the light-induced structural changes had any effect on function, irradiated and nonirradiated samples of Fn and the 40-kDa GBF were compared with respect to their ability to bind to gelatin−Sepharose. The results are presented in Figure 2. Nonirradiated samples of both proteins bound quantitatively and were eluted with 4 M urea; the amount of protein detected in the nonbinding fraction was less than 6% for Fn (Figure 2A) and 0% for 40-kDa GBF (Figure 2B). Exposure to 280-nm radiation for only 10 min increased the nonbound fraction to 35% for Fn and 48% for the fragment. Further irradiation for a total of 60 min increased the nonbound fractions to 46% and >90%, respectively. These results indicate that the structural changes detected by fluorescence are accompanied by a loss of function.

Failure of an irradiated sample to bind to gelatin−Sepharose indicates that its affinity has decreased below a threshold value. It is conceivable that lesser changes in affinity could occur without destroying the ability to bind in this solid-phase assay. To address this possibility, the various samples were tested in a fluid-phase assay, which measures changes in the fluorescence polarization of a labeled collagen fragment, TR-CB7. Dissociation constants obtained in this manner for native pFn and the nonirradiated 40-kDa GBF have been previously reported as ~6.0 and ~25 nM, respectively (Miles & Smith, 1993). However, as shown in Figure 3, 20 min of continuous exposure to 280-nm light increased the apparent $K_d$'s by more than 20- and 50-fold for pFn ($K_d$ = 143 nM; Figure 3A) and 40-kDa GBF ($K_d$ = 1250 nM; Figure 3B), respectively.

*Cleavage of Disulfide Bonds.* It was previously shown by Issacs et al. (1989) that the reduction of disulfides in a 42-kDa GBF produced changes in fluorescence similar to the photoinduced changes described above. The 21-kDa fragment exhibits an unusually large increase in its mobility on SDS−PAGE following the reduction of disulfide bonds (Ingham et al., 1989). We therefore used SDS−PAGE as a first test of whether exposure to 280-nm radiation might result in the cleavage of disulfide bonds. Lanes 1 and 2 of Figure 4 illustrate the effect of chemical reduction on the mobility of 21-kDa GBF. Whereas the native form migrates

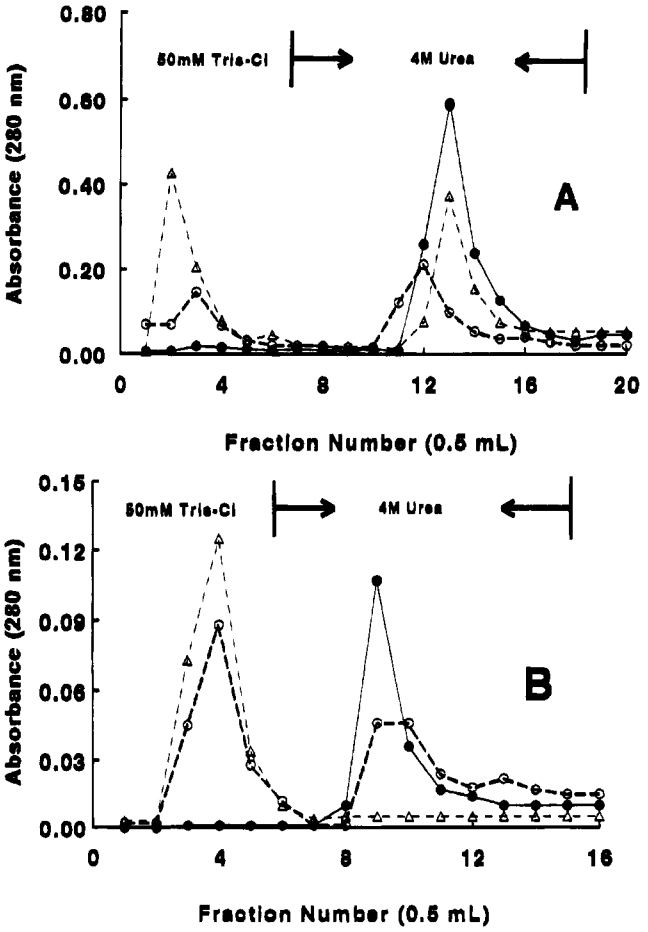


FIGURE 2: Effect of 280-nm irradiation on binding of fibronectin and its 40-kDa gelatin-binding fragment to gelatin–sepharose. Solutions of 3.6 $\mu$M pFn (panel A) or 6.5 $\mu$M 40-kDa GBF (Panel B) were exposed to 280-nm light for 0 (●), 10 (○), and 60 (△) min and applied to 1.0-mL gelatin–sepharose columns equilibrated in 50 mM Tris-Cl, pH 7.4. Bound protein was eluted with 4 M urea in the same buffer. The presence of protein in 0.5-mL fractions was monitored by absorbance at 280 nm.

with an apparent $M_r$ ~24 kDa, the reduced molecule migrates with an apparent $M_r$ of ~21 kDa (hence its name). The samples in lanes 3 and 4 were irradiated for 17 h prior to preparation for SDS–PAGE. Note that the bulk of the nonreduced sample (lane 4) migrates with the same mobility as the reduced sample (lane 3), strongly suggesting that at least those disulfide bonds that are critical for the change in mobility had been reduced as a result of the UV exposure. However, it is conceivable that other bonds could have been broken to cause the same effect. Note that a minor component of the nonreduced sample migrates with $M_r$ close to 40 kDa, suggesting the presence of a reducible dimer whose formation could be mediated by the generation of free sulfhydryls. To determine if disulfides had been severed, samples of nonirradiated and irradiated Fn and fragments were analyzed for their free sulfhydryl content. Table 2 summarizes the results. It is well-known that native pFn possesses 2 free sulfhydryls/250-kDa polypeptide subunit, one in domain $III_7$ and the other in domain $III_{15}$ (Skorstengaard et al., 1986). Both are cryptic in that they react poorly with sulfhydryl-specific reagents except in the presence of denaturing agents (Smith et al., 1982). Accordingly, less than 1 sulfhydryl/mol of native pFn was detected, and a negligible number were detected for the 40- and 21-kDa fragments. However, following the exposure to 280-nm radiation for 20 min, the number of sulfhydryls increased to ~5.8 for fibronectin, to ~4.4 for the 40-kDa, and to ~1.6 for the 21-kDa fragments, detectable without the need for denaturants.

Solutions of pFn and the 40-kDa fragment were also irradiated in the presence of 5 M GdmCl, pH 7.4, and analyzed for sulfhydryl production. The results of these experiments are also reported in Table 2. While the number of photoinduced sulfhydryls detected for Fn decreased slightly, those in the 40-kDa fragment decreased by almost one half. These data suggest a GdmCl-induced change in the structure of the 40-kDa GBF, which otherwise facilitates more extensive disulfide quenching of Trp fluorescence and energy transfer to disulfides.

## DISCUSSION

We have demonstrated that prolonged 280-nm irradiation of pFn or its gelatin-binding fragments causes a time-dependent loss of tertiary structure associated with decreased affinity for gelatin. The structural changes are manifested by a large increase in the intensity and shift to longer wavelength of the intrinsic tryptophan fluorescence. Similar changes can be induced in the 40-kDa fragment by denaturing with GdmCl (Isaacs et al., 1989; Miles & Smith, 1993). The basis of the lower fluorescence of the native protein is not known, but a reasonable possibility is the quenching by disulfide bonds, both of which, according to the known three-dimensional structures of type I domains 4 and 5 (Williams et al., 1994) and domain 7 (Baron et al., 1990) as well as a homologous domain in tissue plasminogen activator (Downing et al., 1992), are in close proximity to conserved Tyr and Trp residues. All of the proposed mechanisms of quenching by disulfides require close contact with the aromatic group (Cowgill, 1976). Visualization of the structure of domain $I_7$ using coordinates obtained from Dr. Ian Campbell (Baron et al., 1990) reveals that both of its indole rings are buried in the core between the $\beta$ sheets, making van der Waals contact with each other and with one or both disulfides. A conserved tyrosine is also in close proximity and could also be quenched. The extent to which this occurs in unknown since Tyr fluorescence in proteins is weak and masked by the stronger Trp fluorescence.

That disulfides affect the fluorescence of type I domains is evident from the fact that their cleavage with reducing agents produces large increases in fluorescence (Isaacs et al., 1989; Ingham & Brew, 1992). This in itself does not prove direct quenching by disulfides since the latter could simply help to hold the Trp residues in close contact with other quenching groups in the protein. Better evidence for a direct interaction between Trps and disulfides came from the observation that prolonged illumination at 280 nm resulted in the breaking of disulfides to produce free sulfhydryls. This undoubtedly involves the transfer of excitation energy to the disulfide bond, which then decomposes with sufficiently high quantum yield to allow chemical detection of free sulfhydryls after only 20-min exposure in the fluorometer. Such cleavage, like that produced with reducing agents, would destabilize the tertiary structure, disrupt the contacts between disulfides and aromatics, and enhance the fluorescence. Cleavage of disulfides could also occur by direct excitation, but the yield would be low due to the low extinction coefficient (Edelhoch, 1967).

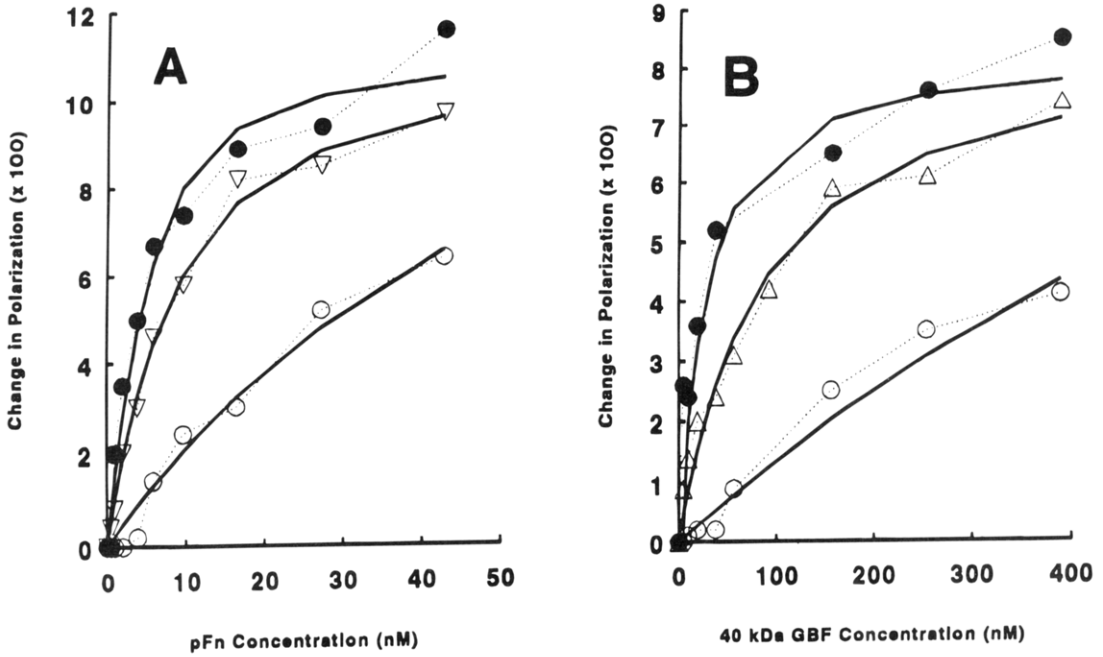


FIGURE 3: Effect of 280-nm irradiation on the $K_d$'s for binding of fibronectin (panel A) and its 40-kDa gelatin-binding fragment (panel B) to the fluorescent-labeled collagen fragment, TR-CB7. Solutions of pFn or the fragment in TBS, pH 7.4, were exposed to 280-nm light in a 1.0-cm quartz cuvette at 25 °C. Aliquots were removed at time intervals of 10 (△) and 20 (○) min and used to titrate TR-CB7 in fluorescence polarization binding assays. Titrations performed using nonirradiated (●) pFn and 40-kDa GBF are also shown. Solid lines were drawn using data obtained from the nonlinear regression analysis of titration data via the Enzfitter computer program (Leatherbarrow, 1987). Apparent $K_d$'s were calculated as described in Miles and Smith (1993).

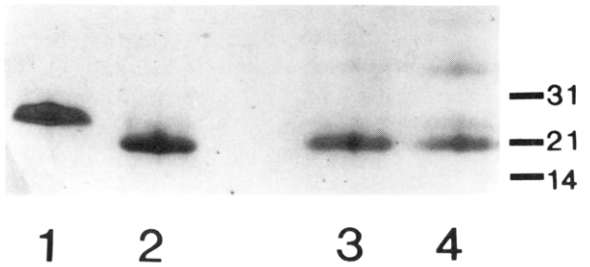


FIGURE 4: Electrophoretic analysis of nonirradiated and 280-nm irradiated 21-kDa GBF. Samples were electrophoresed on 8−25% gradient gels in the presence of SDS. Lanes 1 and 2 contain nonirradiated 21-KDa GBF, nonreduced, and reduced, respectively. A separate sample was exposed to 280-nm radiation for 17 h and then electrophoresed with (lane 3) or without (lane 4) reduction. The positions of molecular weight markers are indicated on the right.

Table 2: Detection of Sulfhydryls in Nonirradiated and 280-nm Irradiated pFn, 40 kDa, and 21-kDa GBF.

| | no. of sulfhydryls detected | | |
|---|---|---|---|
| sample | nonirradiated | irradiated | irradiated in 5 M GdmCl |
| pFn | 0.8, 1.0 | 5.8 | 5.2 |
| 40-kDa GBF | 0.2, 0.2 | 5.4, 4.4, 4.2, 3.9 | 2.6 |
| 21-kDa GBF | 0.0 | 1.6, 1.5 | ND[a] |

[a] ND, not determined.

A glance at Table 1 shows that the greatest effect on fluorescence occurred with 21-kDa GBF. This fragment contains three conserved Trp residues, one in the large loop (refer to Figure 5) between the 1st and 2nd Cys (position 1) and two in the small loop between Cys 3 and Cys 4 (position 2). The other fragments that contain exclusively type I domains are the 29- and 19-kDa fragments. While they also exhibit an increase in fluorescence, the effect is much smaller than for 21 kDa. Only two of eight Trp residues in 29-kDa fragment are located in the small loop. Furthermore, this fragment contains two nonconserved Trps whose position in space is not established and whose fluorescence may tend to partially mask the photochemical effect. Similarly, the 19-kDa fragment, whose fluorescence changes even less, has only one of its five Trp residues in loop 1 and also contains a nonconserved Trp in the linker region between type I domains 10 and 11. The 13-kDa GBF, which exhibits yet a smaller increase has none of its Trps in loop 1. These correlations provide some indication that conserved Trps in the small loop may be the primary contributors to the observed phenomena. Although both Trps in domain $I_7$ are close to disulfides, their orientations are quite different. Further study of the effects of UV irradiation on the structure of domain $I_7$ and mutants thereof might help to clarify the nature of the quenching phenomenon.

Recently reported spectral measurements have demonstrated significant agreement between yearly decreases in stratospheric ozone and increased intensity of UVB (280−320-nm) radiation (Kerr & McElroy, 1993). Certain aquatic ecosystems are deleteriously affected when overexposed to UVB radiation (Bothwell et al., 1994).

The loss of protein−protein interaction induced by irradiation with 280-nm light could also have important consequences *in vivo*. The extent to which fibronectin binds to native collagen is still unclear, but both proteins are abundant in human skin where they are found in association (Vaheri et al., 1978; Fleischmajer & Timpl, 1984). Fibronectin also binds proteoglycans (Hedman et al., 1982), and both collagen (Schwarz et al., 1993) and proteoglycans (Margelin et al., 1993) are reported to be altered in UV-irradiated skin. Exposure to UVB light from solar radiation could disrupt the structure of type I domains in Fn, weakening their interactions with these molecules and

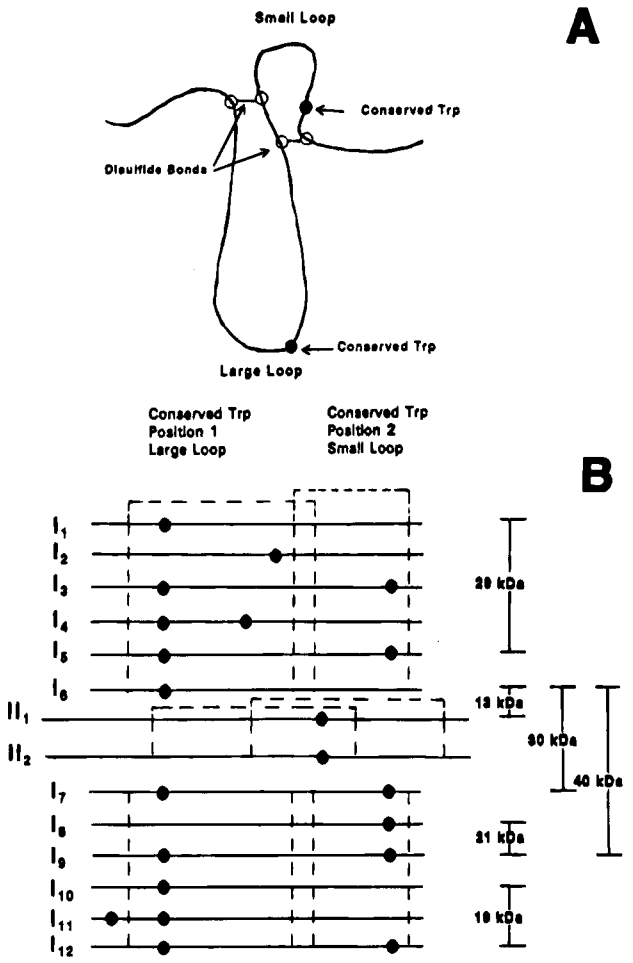


FIGURE 5: Schematic diagram of a Fn type I repeat module (panel A) and the location of Trps in fibronectin type I and type II repeat module (panel B). The dotted lines indicate the approximate position of the two disulfide loops.

contributing to the erythema and blistering of human skin which occurs in sunburn (Ambach & Blumthaler, 1993). This phenomenon may occur in other sun-exposed animal biosystems, perhaps aggravated by diminishing stratospheric ozone levels (Blaustein & Wake, 1995).

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