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Effect of UV irradiation on type I collagen fibril formation in neutral collagen solutions

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Background: **Collagens have the well-known ability to spontaneously self-associate to form fibrils at physiological temperature and neutral pH *in vitro* and *in vivo*. Because solar UV may photochemically alter collagen, the kinetics of fibril formation may be modified. Thus, we have begun a systematic study of the effect of various UV wavebands on fibril formation.**
Methods: **Citrate-soluble calf skin collagen (Elastin Products) was dissolved at 0.05% in 0.5 M HOAc, dialyzed over 2 days into two changes of 0.0327 M phosphate buffer, pH 7.0 at 4°C, and centrifuged at 48 000×*g*. Photolysis was carried out at 4°C with either (a) UVC (UVG–11 lamp), (b) filtered solar-simulating radiation (SSR) or UVA (SSR or UVL–21 lamp filtered with a 2.0 mm Schott WG 345 filter). Gelation was commenced by rapidly raising the temperature from 8°C to 33°C. *Nucleation* and *growth* were followed by turbidimetric measurements at 400 nm.**
***Results*: UVC radiation (0–17.3 J/cm^2) resulted in a dose-dependent decrease in the rate of fibril growth. Under these conditions, concomitant collagen cross-linking and degradation occurred. Fibril nucleation, a prerequisite for growth, was rapid (threshold ~ 2 min) and was not affected by UVC, UVA or SSR. SSR (0–1320 J/cm^2) caused a small decrease in growth rate and in the degree of fibril formation. UVA radiation (0–1080 J/cm^2) had a similar effect. "Direct" photochemical damage thus paralleled absorption via various collagen chromophores, with UVC>SSR~UVA. The presence of riboflavin (RF) resulted in ground-state interactions that markedly altered both nucleation and growth kinetics. Irradiation with 29.6 J/cm^2 UVA in the presence of RF photosensitizer caused relatively minor additional changes in fibrillation kinetics.**
Conclusions: **These results collectively indicate that fibril formation is markedly dependent on specific ground state interactions and relatively insensitive to nonspecific UV damage. On the other hand, fibrils thus formed from photochemically altered collagen may have altered structural properties that could have subtle but unfavorable effects on the local dermal milieu *in vivo*. Notwithstanding, the relative insensitivity of fibrillogenesis to non-specific photochemical damage probably represents a favorable adaptation, overall, which tends to conserve the mechanical integrity of the skin.**

Key words: **collagen; fibrillation; ultraviolet radiation.**

Collagen fibers are the principal structural element of the extracellular matrix (ECM) *in vivo*. In addition, they provide a specific milieu for surrounding fibroblasts and other cells. Remodeling of the ECM is a prominent feature in wound healing, tissue development, tumor invasion, chronic inflammation and other processes (see (1) and references therein). Depending on the degree of mechanical stress the ECM is under, the resident fibroblasts may differ markedly in phenotype, morphology, proliferative capacity and collagenase biosynthesis (2). High stress (e.g. granulation tissue in wounds) favors cell proliferation and biosynthetic activity whereas low stress (intact dermis) favors a non-proliferative phenotype and decreased response to growth factors (2). Perturbations wrought by physical, chemical and/or environmental agents, if severe enough, result in "wound healing" responses by the resident fibroblasts, with attendant changes in ECM structural scaffolding. As a consequence, fibril structure and, by extension, the kinetics of fibril formation could be significantly altered.

Collagen is also well-known to spontaneously self-associate *in vitro* to form *fibrils* at physiological temperatures and neutral pH in an entropy-driven process (4). These fibrils resemble those found in native connective tissue (3, 4). The overall kinetics of fibril formation are determined by two parameters: *nucleation* and *growth.* Ground state interactions between small molecules and collagen at specific sites are known to markedly affect these parameters (2). Photochemical transformations may also be manifested as altered fibrillation kinetics. Miyata et al. (5) and Sudoh & Noda (6) reported suppression of fibril formation under UVC irradiation. Under these conditions, they attributed the major portion of damage to photochemical disruption of peptide bonds and to photomodification of aromatic residues in the telopeptide portion of the collagen molecule. Modest amounts of UV ($\leq$3.0 J/cm^2) at wavelengths longer than 260 nm had little apparent effect (6).

Previous observations that both UVA (7, 8) and UVB (9) stimulate production of matrix metalloproteinases convinced us to more thoroughly investigate the effect of *solar* UV wavelengths on fibril nucleation and growth, using high doses of solar-simulating radiation (SSR) and UVA. In addition, we have sensitized UVA photolysis of collagen with the "physiological" riboflavin (RF). We obtained measurable damage in all cases, with UVC>>SSR$\sim$UVA. We also present evidence for specific ground state interaction between RF and collagen that must occur at a site close to that which determines fibrillogenesis.

Material and methods

Reagents

Except where otherwise noted, all chemicals were obtained either from Fisher Scientific Co. (Norcross, GA, USA), or Sigma-Aldrich (Saint Louis, MO, USA). These were reagent grade and therefore used without further purification. Citrate-soluble collagen was obtained from Elastin Products, Owensville, MO, USA. It was treated as described below.

Collagen sample preparation

All work was done at 4°C. Citrate-soluble collagen was dissolved at 0.5 mg/ml in 0.5 M HOAc and dialyzed over 2 days against two changes of 100 volumes of 0.0327 M phosphate buffer, pH 7.0. The precipitate that forms in the cold was removed by centrifugation at 48 000$\times g$ for 30 min. Protein concentration was estimated from hydroxyproline measurements, as determined by HPLC of a hydrolyzed aliquot. Recovery was generally 73–80 %, corresponding to collagen concentrations ranging from 0.37 to 0.40 mg/ml. All gelations were carried out in 0.0327 M phosphate buffer, pH 7.0. Fresh solutions were made prior to each experiment.

Thermal gelation–fibril formation

All thermal gelations were carried out in a Perkin-Elmer Lambda 3B UV-Visible Spectrophotometer using a thermostatted 1.0 cm cuvette (model #160-QS, Hellma Cells, Plainview, NY, USA). Temperature change in the cuvette was effected by switching from a circulating bath (Lauda, K-2/R; Brinkmann Instruments, Inc., Westbury, NY, USA) set at 8°C to a circulating bath set at 33°C by means of three-way stopcocks. Re-equilibration took approximately 1 min.

A 1.4 ml aliquot (un)irradiated collagen sample was pre-equilibrated to 8°C for 5 min in the thermostatted cuvette. Thermal self-assembly to form fibrils was monitored by continuously recording the optical density at 400 nm as a function of time. Nucleation was assessed by measuring the threshold (lag time) for increase in OD_{400}, denoted by t_o. Fibril growth was sigmoidal, with an initial (approximately linear) rapid rise in OD_{400}, followed by an asymptotic approach to a maximum (OD_{max}). Under conditions of constant pH, concentration and ionic strength, the size of the fibril is proportional to OD_{max} (3). Except where otherwise indicated, results are reported as mean$\pm$S.D.

Fibril growth rate was assessed by measuring the initial slope of the growth curve. The data were remarkably self-consistent over the course of these experiments; the overall result of 17 measurements taken over 5 months afforded *slopes* of 0.102$\pm$0.023 OD/min, with t_o=1.8$\pm$0.4 min, and $OD_{max.}$=0.271$\pm$0.045. To better detect small differences resulting from some of the various UV treatments, the treated groups were always compared with an untreated control from the same sample run the same day.

Light sources

Total radiation output was measured with an Eppley thermopile in conjunction with a Keithley Model 195A digital multimeter. Spectroradiometric measurement of the sources was performed using a model OL-754 Spectroradiometer (Optronic Laboratories) calibrated with a quartz halogen standard traceable to NIST. Measurements were made at use distances, 250–450 nm at 1 nm increments with a band pass of approximately 1 nm.

UVC radiation was produced by a 4 watt UVG–11 shortwave handlamp (Ultraviolet Products), which emits primarily at 254 nm (solid line in Fig. 1a). A 4 watt UVL–21 longwave handlamp (Ultraviolet Products) in conjunction with a 2.0 mm Schott WG 345 filter produced broadband UVA radiation between 345 and 366 nm, and no detectable emission less than 335 nm (dotted line in Fig. 1a). This low intensity UVA source was used for the

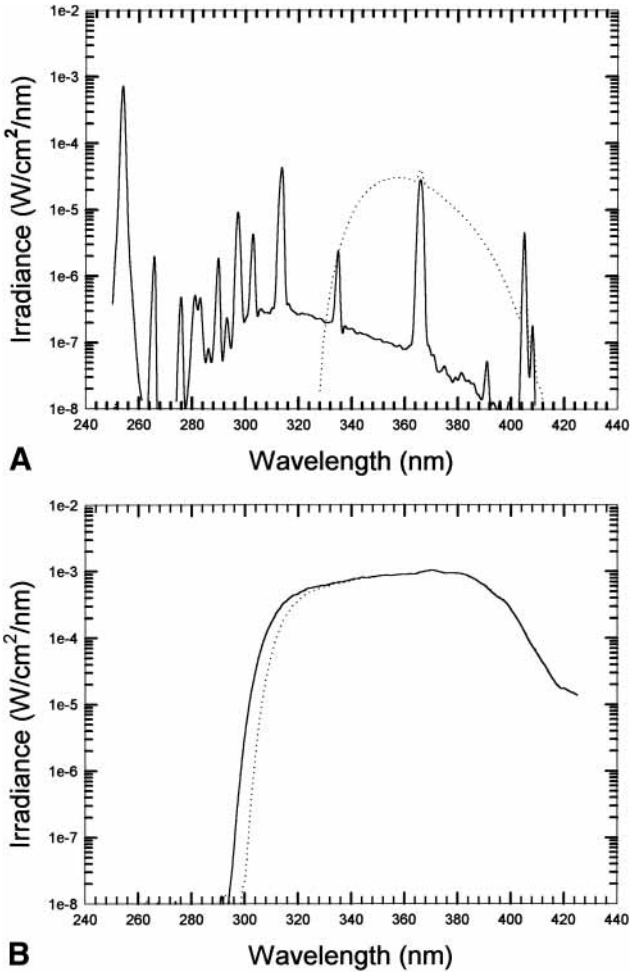

Fig. 1. (A) Spectrum of mercury sources. Solid line: germicidal 4 Watt UVG–11 source. This source emits primarily 254 nm, with roughly 10% of the radiation greater than 300 nm. Dashed line: 4 Watt UVL–21 filtered with 2 mmWG-345 source. The WG-345 filter is used to avoid possible confounding results due to the normal presence of mercury lines at 254, 297, 303, 313 nm. (B) Spectrum of 1.6 kW Xenon Arc Solar Simulator (Source of SSR). Solid line: solar simulator filtered with 1 mm WG-320 and 2 mm Corning #9863. Dashed line: solar simulator as in A filtered with additional 2 mm WG-345. This serves as a high intensity source of UVA.

riboflavin sensitization experiments. In other experiments, a 1.6 kW xenon arc solar simulator produced broadband solar-simulating UV radiation (SSR). A high intensity UVA source was derived from using the solar-simulator in conjunction with the 2.0 mm Schott WG 345 filter. This latter source was used for direct UVA photolysis in the absence of riboflavin sensitizer. Spectral distributions for these high intensity sources are shown in Fig. 1b.

Direct UV irradiation of collagen

Prior to gelation, aerated collagen solutions were irradiated at 0–4°C with various wavebands of UV from the sources described above. Radiation doses are corrected for absorption and reflection by the Pyrex beaker in the cases where the filtered or full-spectrum solar-simulated radiation was used. When the low intensity UVC and UVA lamps were used, the collagen was irradiated directly in the quartz gelation cuvette while equilibrated at 4°C with the "cold" temperature bath. When the solar simulator was used, the irradiated solution was kept cool by means of immersing the reaction cuvette in ice water contained in a Pyrex beaker. This filtered out some of the UVB (51% transmission at 300 nm; 26% transmission at 290 nm). Fluence rates for these sources were 1.11 mW/cm^2 for the UVG–11, 1.97 mW/cm^2 for the filtered UVL–21, 135.0 mW/cm^2 for the "complete" SSR spectrum produced by the solar-simulator and 115.0 mW/cm^2 for the Schott WG 345-filtered solar simulator. Working distances were 2.5 cm for the UVG–11 and UVL–21 sources and 5 cm for the solar simulator.

Riboflavin-sensitized UVA irradiation of collagen

Riboflavin (RF) was added to collagen solutions in the dark at 4°C at final concentrations of 0.7, 1.4 or 14 μM in the gelation apparatus. Dark controls were immediately gelled as before. Test samples were irradiated at 4°C with the filtered UVL–21 source with 30 J/cm^2 UVA prior to gelation.

Results

Gelation

Prepared type I collagen solutions were clear to the eye, and showed little or no turbidity at 8°C, as judged by OD_{400}. The centrifuged solutions showed significantly less intermolecular cross-linking by SDS PAGE than did the uncentrifuged solutions. However, there remained a considerable degree of cross-linking even after centrifugation.

When the temperature of these collagen solutions was rapidly raised from 8°C to 33°C, fibril formation rapidly occurred, with t_o=1.8+0.4 min (n=17), much shorter than those reported by other authors (4). Gelation was not totally reversible in our system. Fibril growth was sigmoidal and was most rapid for unirradiated solutions (Figs. 2–6).

Effect of UV irradiation on fibril formation

UVC radiation: UVC irradiation (0–8 J/cm^2) only marginally affected nucleation; (t_o=2.3±0.4 minutes; n=9) but resulted in a significant dose-dependent decrease in the rate of fibril growth (Fig. 2). A UVC dose of 8.0 J/cm^2 (120 min irradiation) reduced the rate to 0.012 OD/min; OD_{max}=0.050 (n=3).

Solar simulating radiation (SSR): Irradiation with 1460 J/cm^2 (180 min) solar-simulating radiation produced a

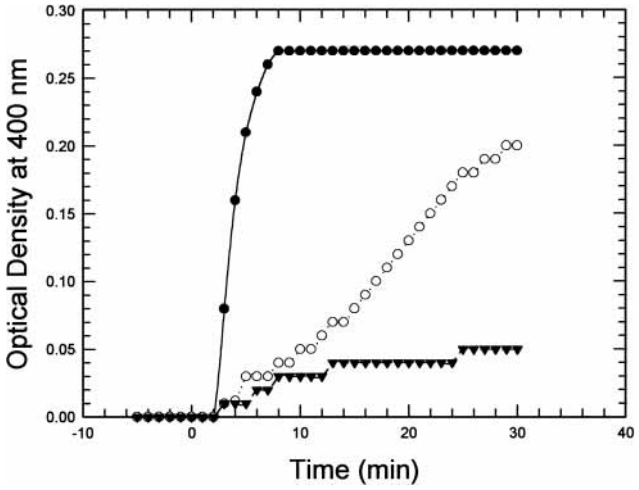

Fig. 2. Effect of UVC radiation on type I collagen fibril formation in 0.0327 M phosphate buffer, pH 7.0. Closed circles: unirradiated control. Open circles: 120 min radiation with 4 W UVG –11 lamp (5.3 J/cm^2). Solid triangles: 180 min radiation with UVG –11 lamp (8.0 J/cm^2).

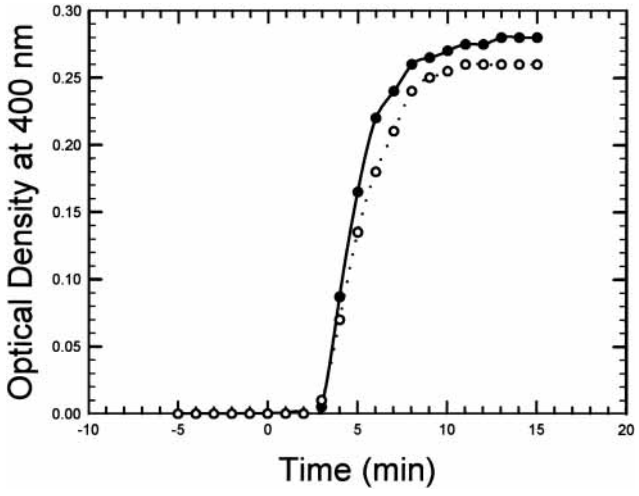

Fig. 3. Effect of SSR on type I collagen fibril formation in 0.0327 M phosphate buffer, pH 7.0. Closed circles: unirradiated control. Open circles: 120 min radiation with 1.6 kW xenon arc solar simulator (973 J/cm^2).

marginal change in nucleation; t_o=2.2+0.4 min (n=8). A small decrease in growth rate was observed (0.098 OD/min for the control to 0.078±0.024 OD/min (Fig. 3). This decrease was not statistically significant, but was felt to represent a trend.

UVA radiation: Irradiation with 623 J/cm^2 UVA produced a small change in nucleation, with t_o=2.3±0.5 min (n=3). Again, a slight decrease in growth rate was observed (OD/min=0.081±0.033) that was not statistically significant (Fig. 4).

Ground-state interaction between riboflavin (RF) and collagen: Since direct irradiation of collagen with solar wavelengths did not lead to striking alterations in fibrillation kinetics, we carried out UVA-photosensitization with riboflavin (vitamin B_2), known (10) to cause considerable damage to collagen. When riboflavin (RF) was added to collagen in the dark at 8°C, there was a concentration-

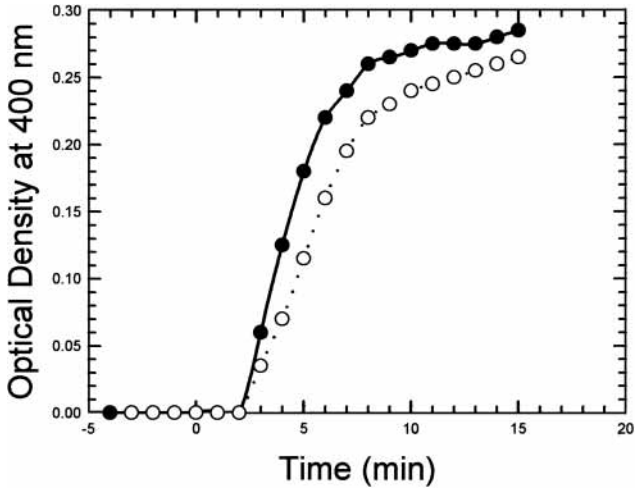

Fig. 4. Effect of UVA on type I collagen fibril formation in 0.0327 M phosphate buffer, pH 7.0. Closed circles: unirradiated control. Open circles: 90 min radiation with UVA from a 1.6 kW xenon arc solar simulator plus 2 mm Schott WG 345 filter (623 J/cm^2).

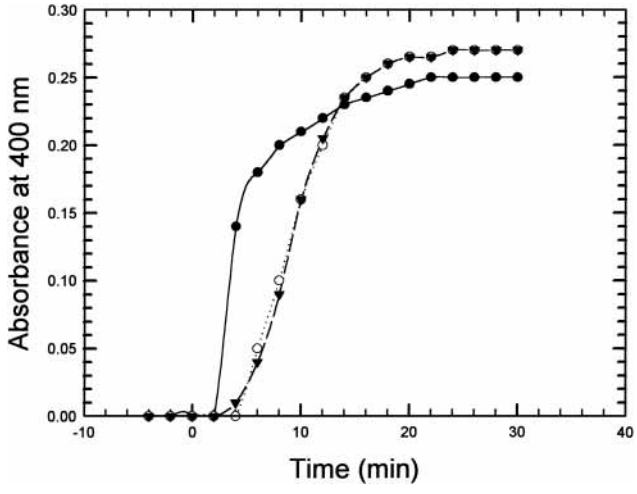

Fig. 5. Ground-state interaction between riboflavin (RF) and type I collagen: effect of UVA on type I collagen fibril formation in 0.0327 M phosphate buffer, pH 7.0 Closed circles: unirradiated control. Open circles: unirradiated mixture of RF (0.7 μM) and collagen. Triangles: mixture of RF (0.7 μM) and collagen irradiated with UVA from a 4 W UVL–21 lamp with 2 mm Schott WG 345 filter (30 J/cm^2).

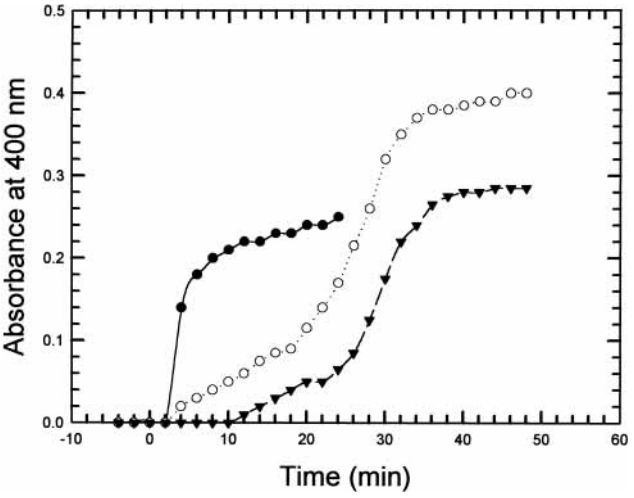

Fig. 6. Ground state interaction between riboflavin (RF) and collagen: effect of UVA on type I collagen fibril formation in 0.0327 M phosphate buffer, pH 7.0 Closed circles: unirradiated control. Open circles: Unirradiated mixture of RF (1.4 μM) and collagen. Triangles: mixture of RF (1.4 μM) and collagen irradiated with UVA from a 4 W UVL–21 lamp with 2 mm Schott WG 345 filter (30 J/cm^2). Mixture of 14 μM RF-collagen yielded the same results (not shown).

dependent change in the kinetics of fibril formation in the absence of UVA. At [RF]=0.7 μM, t_o increased from 2.0 to 5.2 min, and the rate of growth decreased from 0.075 OD/min; OD_{max}=0.235 (n=2) to 0.029±0.002 OD/min (OD_{max}=0.245) as seen in Fig. 5. At [RF]=1.4 μM, a biphasic increase in turbidity, with "slow" and "rapid" growth components, was observed (Fig. 6). Again, the rate of growth of the "rapid growth" component *decreased* to 0.028±0.003 (n=3). However, OD_{max} *increased* to 0.403±0.078. Essentially the same results were obtained at [RF]=14 μM. Evidently, there exists stable ground-state interaction between RF and collagen that results in altered fibrillation kinetics. The rate of fibril formation is retarded, but a larger fibril is produced.

Riboflavin-sensitized UVA photolysis: As indicated by SDS-PAGE (data not shown), RF-sensitized UVA photolysis (30 J/cm^2) caused significant chain degradation and cross-linking, in general agreement with previous results by Carbonare & Pathak (10). Notwithstanding, UVA irradiation of collagen in the presence of 0.7 μM RF produced little or no apparent change in the fibrillation curves (Fig. 5). At [RF]=1.4 μM, 30 J/cm^2 UVA suppressed the "slow growth" phase, while not significantly affecting the "rapid growth" phase (slope=0.023). OD_{max} decreased to 0.305 (n=1), In all cases, RF-sensitized photolysis had no effect on t_o relative to the ground state RF–collagen complex (Fig. 6).

Discussion

Our calf-skin collagen preparation rapidly self-associates to form fibrils when the temperature of the solution is rapidly raised from 8° to 33°. The ability to self-associate is relatively insensitive to UV-induced photochemical damage. The much shorter lag time as well as the lack of reversibility in our system *vis à vis* the salt-soluble preparation of Helseth & Veis (4) is undoubtedly a result of the relatively high degree of cross-linking in our sample compared to theirs. As cross-linking favors self-assembly, it is likely that even photochemically modified collagen molecules will tend to self-assemble as long as they are not too badly degraded, although the packing of UV-damaged fibrils may well be significantly modified. More drastic UVC irradiation causes extensive collagen degradation, and markedly alters fibrillogenesis. Since collagen molecules tend to be highly cross-linked in mammalian skin, it is very probable that the results reported here reflect the situation *in vivo.*

The susceptibility of collagen fibril formation to UV mirrors collagen absorption. The strongest and most obvious absorption is at wavelengths shorter than 300 nm, due mainly to aromatic residues (λ_{max}~275 nm) and peptide bonds (<260 nm). Absorption by solar wavelengths is not as obvious, but may be deduced by the presence of fluorophores with excitation spectra in this region (12). Similarly, the most obvious change in fibrillation kinetics occurs under UVC radiation. Subtle UV-induced alterations in fibrillogenesis occur at solar wavelengths, but these require high UV doses to be discerned. It might be argued that the doses of solar UV used to elicit the small effects observed by us are much too high to have any relevance to the human situation. Given the slow turnover of dermal collagen, however, it is quite possible that cumulative doses of solar UV to these molecules might reach those levels under chronic conditions of exposure.

Fluorescence and SDS-PAGE data indicate significant damage by solar UVB and UVA wavelengths under conditions where the fibrillation appears to be marginally affected. The fluorescence emission spectrum of the collagen preparation used in this study, similar to those previously published for acid extracted mouse collagen (11), include emission maxima at 305 nm (tyrosyl) and 430 nm, as well as shoulders at 315 nm and 360 nm. Irradiation with solar UV wavelengths results in irreversible "fading" of fluorescence at $\lambda \geq 315$ nm and produces some signs of concomitant photochemical cross-linking and chain degradation.

Our UVC results might be directly compared with those of other workers (5, 6). These workers used acid-

soluble collagen preparations from calf skin and rat tail, respectively. Both groups reported higher initial values of t_o, as well as an increase in this parameter on UVC irradiation, in contrast to our results. On the other hand, UVC irradiation caused a marked reduction in fibril growth, which is in qualitative agreement with our observations. There was no indication of the degree of cross-linking in their preparations and their conditions of pH and ionic strength concentration and final temperature, parameters to which fibrillation kinetics are very sensitive (3, 12) were different from ours. Therefore, these differences complicate the direct comparison of our results with theirs. On the other hand Helseth & Veis (4), used similar conditions of pH, temperature, ionic strength and method of preparation, so that in this case, comparison between preparations was more straightforward.

Alterations in fibrillation kinetics wrought by solar UVA and UVB were subtle despite the high doses afforded by our experiments. Therefore, we carried out irradiation with the "physiological" UVA photosensitizer riboflavin, known (10) to cause marked photochemical cross-linking and chain degradation in the presence of UVA wavelengths. To our surprise, we observed marked ground state interaction between RF and collagen, even at [RF]=0.7 μM, much lower than the collagen amino acid residue concentration. This interaction is "saturable", since virtually identical results were obtained at [RF]=1.4 and 14 μM. Specific interactions between the triple helical region of one chain and the telopeptide region of a second chain appear to be responsible for self-assembly (4). Evidently, RF binds collagen at specific sites close enough to the region where the initiation of fibrillogenesis takes place to significantly alter the fibrillogenesis kinetics. On the other hand, irradiation of the RF-collagen mixture with UVA doses that caused marked non-specific cross linking and chain degradation had only minimal effect on the gelation kinetics. Thus, it seems that the ability to self-associate is much more affected by interactions at highly specific loci than by non-specific damage at points removed from these special sites, a conclusion also reached by Helseth & Veis (4). In the case of UVC radiation, on the other hand, extensive peptide scission throughout the collagen molecule as well as photomodification of aromatic amino acid residues in the telopeptide region eventually result in inhibition of fibrillogenesis (5, 6).

As an *in vivo* event, insensitivity to non-specific photodamage by solar wavelengths that can penetrate to the dermis probably represents a favorable adaptation, overall, that tends to conserve the mechanical integrity of the skin. UV-damaged fibers would confer on the dermis a certain mechanical strength that, although not optimal, is nonetheless much greater than would be the case if fibrillation were more greatly inhibited by solar wavelengths. In the latter case, one might expect human skin to be even more adversely affected by solar UV and to exhibit significantly increased fragility, scarring and other signs of "photoaging" at lower doses of irradiation.

On the other hand, the marked alteration of collagen fibrillogenesis by RF in the ground state suggests the possibility that analogous interactions could exist between dermal type I collagen and other ground substance macromolecules (e.g. acid glycosaminoglycans) and/or type III collagen. Thus, other macromolecules might have a pivotal role in helping collagen to provide a dermal milieu that is "optimal" for fibroblasts and other resident cells.

In summary, our results indicate that collagen damaged by *solar* UV wavelengths may still form fibrils, but, due to photochemical molecular alterations, these fibrils may not have the same structure, conformation or local environment as intact collagen. Such photochemical modifications in collagen structure and architecture could conceivably have subtle but unfavorable effects on the local dermal milieu *in vivo* that could lead to altered wound-healing, mechanical, immunological and/or oncogenic properties. However, the very lack of sensitivity of fibrillogenesis to non-specific photochemical damage probably represents a favorable adaptation, overall, that tends to conserve the mechanical integrity of the skin.

References

1. Stricklin GP, Liying L, Vesna J, Wenczak, BA . Localization of m-RNAs representing collagenase and TIMP in sections of healing human burns. Am J Pathol 1993: **143**: 1657–1666.
2. Grinnel F. Fibroblasts, myofibroblasts, and wound contraction. J Cell Biol 1994: **124**: 401–404.
3. Gross J, Kirk D. The heat precipitation of collagen from neutral salt solutions: some rate–regulating factors. J Biol Chem 1958: **233**: 355–360.
4. Helseth DL, Veis A. Collagen self-assembly *in vitro.* J Biol Chem 1981: **256:** 7118–7128.
5. Miyata T, Sohde T, Rubin AL, Stenzel KH. Effects of ultraviolet irradiation on native and telopeptide-poor collagen. Biochim Biophys Acta 1971: **229:** 672–680.
6. Sudoh K, Noda H. UV-Irradiation of collagen and its effect on fibril reconstitution. Connect Tissue Res 1972: **1:** 267–274.
7. Scharffetter K, Wlaschek M, Hogg A, et al. UVA irradiation induces collagenase in human dermal fibroblasts *in vitro* and *in vivo.* Arch Dermatol Res 1991: **283:** 506–511.
8. Petersen MJ, Hansen C, Craig S. Ultraviolet A irradiation stimulates collagenase production in cultured human fibroblasts. J Invest Dermatol 1992: **99:** 440–444.
9. Brenneisen P, Oh J, Wlaschek M, et al. Ultraviolet B wavelength dependence for the regulation of two major matrix-metalloproteinases and their inhibitor in human dermal fibroblasts. Photochem Photobiol 1996: **64:** 877–885.
10. Carbonare MD, Pathak MA. Skin photosensitizing agents and the role of reactive oxygen species in photoaging. J Photochem Photobiol B: Biol. 1992: **14:** 105–124.
11. Menter JM, Williamson GD, Carlyle K, Moore CL, Willis I. Photochemistry of type I acid-soluble calf skin collagen: dependence on excitation wavelength. Photochem Photobiol 1995: **62:** 402–408.

12. Hayashi T, Nagai Y. Factors affecting the interactions of collagen molecules as observed by *in vitro* fibril formation. J Biochem 1973: **74:** 253–262.

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