Identification of an Intermediate State in the Helix-Coil Degradation of Collagen by Ultraviolet Light*

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Differential scanning calorimetry has revealed the presence of a new denaturation endotherm at 32 °C following UV irradiation of collagen, compared with 39 °C for the native triple helix. Kinetic analyses showed that the new peak was a previously unknown intermediate state in the collagen helix-coil transition induced by UV light, and at least 80% of the total collagen was transformed to random chains via this state. Its rate of formation was increased by hydrogen peroxide and inhibited by free radical scavengers. SDS-polyacrylamide gels showed evidence of competing reactions of cross-linking and random primary chain scission. The cross-linking was evident from initial gelling of the collagen solution, but there was no evidence for a dityrosine cross-link. Primary chain scission was confirmed by end group analysis using fluorescamine. Electron microscopy showed that the segment long spacing crystallites formed from the intermediate state were identical to the native molecules. Clearly, collagen can undergo quite extensive damage by cleavage of peptide bonds without disorganizing the triple helical structure. This leads to the formation of a damaged intermediate state prior to degradation of the molecules to short random chains.

Studies of the effect of UV radiation on the properties of the collagen molecule, in solution or in its aggregated fiber form, are rather limited. It has been reported that cross-linking and degradation (1) occur on exposure to UV, the relative proportions depending on the presence of oxygen, pH of solution, type of collagen, and wavelength of the UV.

These effects have been attributed to absorption by the aromatic groups, phenylalanine and tyrosine, with the suggestion that cross-linking could be mediated through dityrosine cross-links (2), although no detailed chemistry has been carried out to demonstrate the presence of this cross-link. For example, Kato et al. (3) reported loss of tyrosine and cross-linking in both type I and IV collagens but could not detect dityrosine, only DOPA.¹ Kaminiska and Sionkowska (4) demonstrated that the infrared amide bands were shifted to a lower frequency, indicating that structural changes were taking place in the molecule. They also deduced that the helix-random coil transitions of collagen solutions (12), fibers (13), and tissues (14). Collagen monomers in solution unfold abruptly to yield a single, sharp, and highly energetic endotherm. We have shown that the thermal helix-coil transition proceeds via a single, first-order rate process (15) in which there is no intermediate state.

In this paper, we report that the UV-induced transition proceeds via a previously unknown intermediate state and discuss the possible mechanisms involved. It was possible to demonstrate minor changes in the structure of the collagen molecule resulting in the intermediate state and follow the progression of cross-linking and degradation. An understanding of the mechanisms involved in these changes and their inhibition is obviously of considerable interest to the effect of UV on the aging of dermal collagen and the use of UV in the treatment of psoriasis, where there is a potential for damage to the underlying collagen fibers.

EXPERIMENTAL PROCEDURES

Collagen Solutions

Collagen solutions were prepared from tendons freshly dissected from frozen rat tails, dissolved in 0.5 M acetic acid, and centrifuged at 10,000 rpm for 20 min to remove undissolved material. The solution was scanned by DSC to determine the enthalpy of the transition per unit volume of the solution, and the concentration of collagen was calculated using a transition enthalpy of 70 J/g (12). On this basis, the solution concentration was adjusted to 2 mg/ml by the addition of 0.5 M acetic acid, prior to UV irradiation.

Ultraviolet Irradiation

Solutions were irradiated using UV-C light from two 6-watt fluorescent lamps (type TUV 6W; Philips, Croydon, Surrey, UK) each specified by the manufacturer to yield an output of 2.1 watts centered at 253.7 nm. The tubes were mounted in a plane with their axes parallel and 4
cm apart. To the rear of the tubes was a cylindrically concave reflecting head (Agar Products Ltd., Stansted, Essex, UK), directing the UV light forward through a 6 × 24-cm rectangular aperture. The collagen solution was held in a quartz cuvette, placed centrally in the beam with its front face positioned 1 cm from the tangential plane connecting the front surfaces of the fluorescent tubes.

**DSC Analysis**

After measured irradiation times, 0.4 ml of solution was taken from the cuvette and diluted to 0.4 mg/ml with 0.5 M acetic acid. The solution was stirred and degassed for 8 min using a Thermovac apparatus (Microcal Inc., Northampton, MA) and scanned in a VP-DSC (Microcal) from 10 to 60 °C. Numerical analysis of the data was undertaken with the Microcal software using a cubic interpolation for the baseline and a "non-two-state" fitting procedure with cursor initiation. To obtain convergence, it was necessary to provide initial estimates of ΔH, on the order of 10^5 cal/mol. Least squares fitting of the nonlinear function (Equation 2) was performed using Origin Software.

**Hydrogen Peroxide**

To investigate the effect of increasing OH radical concentration during UV irradiation, approximately 0.3% hydrogen peroxide (Sigma) was added to the collagen solution prior to irradiation.

**Thiourea and Cysteamine**

To examine the effect of reducing the free radical concentration during UV irradiation, the free radical scavengers, thiourea and cysteamine (Sigma), were added at concentrations of 1, 10, and 100 mM.

**SLS Crystal Formation**

The irradiated solutions were examined to see whether the collagen molecules were still capable of producing SLS crystals and fibers. SLS crystals were prepared as follows. Solutions were diluted to 0.2 mg/ml collagen in 0.05 M acetic acid and dialyzed against 0.4 g of ATP dissolved in 0.05 M acetic acid. The crystallites were examined by transmission electron microscopy (Philips 400). Other subsamples of the crystallites were degassed and run in the VP-DSC, and the collagen content of further samples were measured by hydroxyproline analysis both in the suspensions themselves, the pellet after centrifugation of a specified volume, and the supernatant. The latter tests were done to determine how much of the sample produced the SLS crystals.

**Chemical Analyses**

**Amino Acid Composition**—The composition of the irradiated collagen was determined to investigate any specificity in the degradation of the amino acids. The samples were hydrolyzed in 6 M hydrochloric acid and analyzed on an Alpha Plus II Autoanalyzer (Amersham Pharmacia Biotech) using the standard program, and detection of the amino acids was achieved by postcolumn derivatization using ninhydrin.

**Intermolecular Cross-Links**—The presence of cross-links was determined on the Alpha Plus using a modified gradient as described previously in detail (16).

**Hydroxyproline Assay**—The collagen content was determined by the standard colorimetric assay (17) but employing the continuous system from Chembak based on the method of Grant (18).

**Chemical Synthesis of Dityrosine**

Dityrosine was synthesized by peroxide oxidation of tyrosine according to the method described by Nomura et al. (19).

**End Group Analysis**

Determination of new amino acid end groups exposed on cleavage of the peptide chains was made by fluorescamine (Sigma). 0.2% (w/v) fluorescamine in acetone was added to 0.4 M lithium borate buffer, and fluorescence was read after 1 min using excitation at 390 nm and measuring emission at 475 nm, as described in detail (20). Preliminary measurements with known quantities of glycine (Sigma) were used to calibrate the fluorescence reading in terms of numbers of amide groups.

**Polyacrylamide Gel Electrophoresis**

The molecular weight changes were demonstrated by SDS-polyacrylamide gel electrophoresis (21) followed by staining with Coomassie Blue; the stained gels were scanned using an Agfa Studioscan I flat bed scanner and Adobe Photoshop software; and the image was analyzed using the NIH Image package.

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**RESULTS**

**Denaturation by a Rate Process**—When freshly prepared collagen solutions were examined in the VP-DSC, the well known single sharp helix-coil transition was seen at a temperature that varied linearly with the logarithm of the scanning rate, as expected for a rate process. The width at half-peak height increased linearly with the scanning rate, consistent with the shape of the enthalpy being broadened slightly by the response time of the instrument.

**Formation of an Intermediate State**—When samples were irradiated and aliquots were examined at different irradiation times, a new endotherm at approximately 32 °C appeared in addition to the known peak for collagen at 39 °C (Fig. 1). The new peak grew with time of irradiation while the first peak fell, and at about 1 h the former was the only endotherm present. Further irradiation reduced this peak progressively, and by about 2 h its area was less than 10% of its maximum value. The two peaks, although easily resolvable, overlapped, and to estimate the enthalpy of the two peaks separately it was necessary to use a least squares fitting procedure (Microcal Inc.). This showed a linear relation between the areas of the new and old peaks (Fig. 2), demonstrating that the new peak was being created at the expense of the original triple helical peak. The new peak appeared to be an intermediate state in the degradation of collagen to random coils by UV following Scheme 1.

\[
\begin{align*}
&\quad k_1 \\
\text{Triple helix} &\rightarrow \text{Intermediate state} &\rightarrow \text{Random coil} \\
\end{align*}
\]

**SCHEME 1**

Since the least squares regression line cut the new peak axis at approximately 80% of the enthalpy of the old peak (Table I), we can say that at least 80% of the collagen followed this general scheme. Equally, the enthalpy of the helix coil transition of the intermediate state must be at least 56 J/g, 80% that of the collagen triple helix.

The solution of the simultaneous differential equations governing the above consecutive reactions is given for first order rate processes in textbooks of physical chemistry (22), from which the concentrations \(x\), of triple helix, \(y\), of the intermediate state, and \(z\), of the random coil state, can be written as follows,

\[
x = x_0 e^{-k_1 t} = x_0 e^{-k_2 t}
\]

(Eq. 1)
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\[ y = \frac{x}{k_2 - k_1} \left( e^{k_2t} - e^{k_1t} \right) \]  
\[ z = x_0 \left( 1 + \frac{1}{k_1 - k_2} (k_2 e^{k_2t} - k_1 e^{k_1t}) \right) \]

Experiment and period over which regression was examined & Intercept ± S.E. & Slope ± S.E. & Residual S.D. & \\
A (0 < t < 900 s) & 54.09 ± 1.58 & -0.795 ± 0.038 & 1.97 & \\
B (0 < t < 900 s) & 55.15 ± 3.70 & -0.820 ± 0.082 & 3.45 & \\
C (0 < t < 60 s) & 57.98 ± 1.60 & -0.835 ± 0.035 & 1.39 & \\
A + B + C data pooled & 55.22 ± 1.26 & -0.810 ± 0.029 & 2.24 & \\

\[ y = \frac{x_0 k_1}{k_2 - k_1} \left( e^{k_2t} - e^{k_1t} \right) \] (Eq. 2)
\[ z = x_0 \left( 1 + \frac{1}{k_1 - k_2} (k_2 e^{k_2t} - k_1 e^{k_1t}) \right) \] (Eq. 3)

where \( x_0 \) is the initial concentration of collagen. Eliminating \( t \) from Equations 1 and 2 yields the following relation between \( x \) and \( y \).

\[ y = \frac{k_1 x_0}{k_2 - k_1} \left( \frac{x}{x_0} \right) \left( \frac{1}{x_0} \right) \] (Eq. 4)

Note that a Taylor expansion of this function at \( x = x_0 \) yields a power series,

\[ y = \frac{x - x_0}{x_0} \left( \frac{x}{x_0} \right)^2 \right] + \text{Higher order terms} \] (Eq. 5)

that is approximately linear for small \( (x - x_0)/x_0 \), especially when \( k_2/k_1 \) is small as in this work.

The concentrations were determined calorimetrically using measurements of the areas under the DSC peaks, denoted as follows:  \( Q_{IS} \) for the intermediate state,  \( Q_{TH} \) for the triple helical state, and  \( Q_{TH0} \) for the triple helical state of the unirradiated sample.

Examples of these calorimetric measurements are plotted in Fig. 3. The lines represent least squares fittings of the data to Equations 1–3. Numerical values for the fitted rate constants \( k_1 \) and \( k_2 \) are given under various conditions in Table II.

Several lines of evidence confirmed that the majority of the native molecules were denatured via the intermediate state. First, estimates of the concentrations of the individual components followed closely the time courses predicted by Equations 1–3 (see Fig. 3). Second, analysis showed that the rate constant \( k_1 \) for the degradation of the triple helix determined directly from Equation 1 agreed closely with the rate constant for the formation of the intermediate state, determined independently (from Equation 2; see Table II). Third, the ratio \( k_1/k_2 \) determined either directly using Equation 4 or by individual determinations of \( k_1 \) and \( k_2 \) using Equation 2 agreed within the experimental uncertainty (Table II). Finally, during the initial period of irradiation, when intermediate state degradation was relatively slight, the intermediate state endotherm grew as the native state declined in approximately a 1:1 relationship, as predicted by the Taylor approximation, Equation 5 (see Fig. 2).

When the exact expression was used to fit all of the data pooled, the enthalpy of denaturation of the intermediate state was estimated as \( 70 \pm 5 \) J/g, the same as the native state.

The temperatures declined and the widths of the endotherms at half height increased with irradiation time under all conditions, and partial denaturation by scanning to approximately \( T_{max} \) followed by rapid cooling showed an elevated temperature and narrower width on rescanning (see Fig. 4). This demonstrated that each endotherm contained a distribution of molecules with different stabilities and that this diversity increased with increasing doses of radiation. The temperatures of both peaks were increased by increasing the scanning rate or by reducing the pH.

Gelation—During the initial period of irradiation (0–1 h) the solution gradually gelled, indicating cross-linking. By 1 h, a weak gel had formed, and the solution could no longer be poured from the cuvette and could only be removed with a spatula. Vigorous irradiation reduced the strength of the gel, and by 8 h, the solution was visibly reduced viscosity.

Molecular Weight Change by Gel Electrophoresis—Gel electrophoresis showed a gradual reduction in the \( \alpha \)-band, \( \beta \)-band, and higher bands with UV dose (Fig. 5). After 1 h of irradiation, the individual \( \alpha \)– or \( \beta \)-bands were barely discernible although the SDS and heat treatment had dissolved all of the collagen.
thermally than the controls (Table III).

showed that the crystals of irradiated collagen were less stable
less ordered internal architecture (Fig. 6). The DSC study
formed from unirradiated collagen (Table III) but possessed a
showed that these crystals were of the same length as crystals
scope (Table III). Measurements of the electron micrographs
revealed the presence of SLS crystals in the electron micro-
mine whether the intermediate state of the irradiated collagen
appeared from the gels (Fig. 5).

DSC thermograms of collagen solution (diluted to 0.4 mg/ml) in 0.5 M
acetic acid after irradiation for 1 h at a collagen concentration of 2
mg/ml and at a temperature of 0 °C are shown. All scans were at
60 °C/h. Peak maxima are indicated in degrees centigrade. Sample was
scanned from 10 to 60 °C and immediately cooled to 10 °C (a) and then
rescanned (b). A new sample was scanned from 10 to 30 °C and imme-
 diately cooled to 10 °C (c) and then rescanned from 10 to 60 °C and
immediately cooled (d) and rescanned (e).

Samples exposed to UV for 1 h or longer showed a continuum of dye along the whole length of the gel with a concentration at the high molecular weight end (Fig. 5a). Careful examination at low UV exposures (5 min) revealed some new bands, below the α-bands, indicating that chain scission may not be entirely random (high density gels, not shown).

Examination for higher molecular weight components, con-
sistent with gelling of the solution, revealed an initial increase in high molecular weight components at the top of the gel, indicating extensively cross-linked α-chains, followed by a sub-
sequent decline. It was concluded that two processes were
taking place simultaneously. The chains had cross-linked but
had also been cut predominantly randomly along their length
so that the resulting population of chain lengths was distrib-
uted down the length of the gel.

Chain Cleavage Determined by Fluorescamine End Group Analysis—The fluor

examine whether the intermediate state of the irradiated collagen
was sufficiently intact to be capable of forming SLS crystals
revealed the presence of SLS crystals in the electron micro-
scope (Table III). Measurements of the electron micrographs
showed that these crystals were of the same length as crystals
formed from unirradiated collagen (Table III) but possessed a
less ordered internal architecture (Fig. 6). The DSC study
showed that the crystals of irradiated collagen were less stable
thermally than the controls (Table III).

The rate constants $k_1$ and $k_2$ ($s^{-1}$) estimated in different ways and under different conditions

| Experiment | $k_1$ from logarithmic form of Equation 1 | $k_2/k_1$ from Equation 4 | $k_1$ from Equation 2 | $k_2$ from Equation 2 | $k_2/k_1$ from previous two columns |
|------------|-----------------------------------------|--------------------------|----------------------|----------------------|-----------------------------------|
| A          | 0.0020999 ± 0.000057                    | 0.10085 ± 0.01085        | 0.001558 ± 0.000137  | 0.000200 ± 0.000015  | 0.1266 ± 0.0205                   |
| B          | 0.001748 ± 0.00016                      | 0.15569 ± 0.03187        | 0.00183 ± 0.000165   | 0.000533 ± 0.000035  | 0.1820 ± 0.0355                   |
| C          | 0.02694 ± 0.00274                      | 0.26397 ± 0.02427        | 0.02603 ± 0.00496    | 0.00899 ± 0.00116    | 0.3454 ± 0.109                    |

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Fig. 4. Evidence that the intermediate state is heterogeneous.

Experiments were as follows: A, collagen solution (2 mg/ml) in 0.5 M
acetic acid at 0 °C; B, collagen solution (2 mg/ml) in 0.5 M acetic acid at room
temperature; C, collagen solution (2 mg/ml) in 0.5 M acetic acid and 0.3% hydrogen peroxide at room temperature.

Acceleration and Inhibition of Hydroxyl Radicals—The addition of hydrogen peroxide to the solution increased both $k_1$ and $k_2$ by over 10-fold. This showed that increasing the concentration of OH· radicals, which are produced by the splitting of the H$_2$O$_2$ molecule when illuminated by ultraviolet light (23), increased both the rate of formation of the intermediate state and
its degradation into random coils (see Fig. 7, Table II). Equally, the addition of the free radical scavengers, cysteamine or thiourea, reduced both $k_1$ and $k_2$. Thiourea was particularly effective and at a concentration of 100 mM suppressed all intermediate state production under the conditions of these experiments (Fig. 8). There was an approximately linear relation between log $k_1$ and log $k_2$ (Fig. 9).

**Chemical Composition**—Amino acid analysis revealed a rapid loss of tyrosine (50% in 1 h) and an even more rapid loss of phenylalanine (70% in 1 h), while other amino acids were virtually unaffected.

**Formation of Unknowns**—We could not detect dityrosine, using an authentic sample synthesized in the laboratory, or DOPA (Sigma), both of which have previously been reported to be present in irradiated collagen (2, 3). Two unknown peaks were present in the “cross-link region” of the chromatogram between tyrosine and hydroxylysine, and both increased with time of irradiation (Fig. 10). Attempts are currently being made to isolate and characterize these two components.

**DISCUSSION**

We have shown that ultraviolet light reduces the native triple helical collagen to random chains via an intermediate state of slightly lower thermal stability, at a $T_m$ of around 32 instead of 39 °C.

The high enthalpy of denaturation and the highly cooperative thermal unfolding of the intermediate state are characteristic features of the triple helix and are therefore consistent with the intermediate state being mainly triple helical. Since the gels showed loss of both $\alpha$- and $\beta$-bands together, we interpret the loss as being caused by chain scission. The gel analysis (Fig. 5) revealed that no intact $\alpha$-chains remained after 1 h of irradiation, when about 60% of the collagen was in the intermediate state (Fig. 3). Chain scission is known to destabilize collagen (24). Thus, we suggest that the intermediate state is a triple helix destabilized, at least partly, by UV-induced chain scission. While the triple helix remains intact, due to hydrogen bonding, a small number of chain scissions would be expected to cause little change in the enthalpy of denaturation, since most of the interchain hydrogen bonds would remain unaffected. The reduction in denaturation temperature would be caused mainly by entropic effects. Irradiated preparations, containing only intermediate state with very little native triple helix, still produced high yields of SLS crystallites, confirming that the intermediate state was triple helical and that the

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**Fig. 6.** Electron micrographs of SLS crystals prepared from collagen solutions before and after 1-h UV-C irradiation. a, un-irradiated solution (i.e., native state). b, solution irradiated for 1 h (i.e., intermediate state and random chains). Bars, 64 nm.

**Fig. 7.** UV-C degradation of collagen solutions (2 mg/ml) in the presence of 0.3% hydrogen peroxide. Numbers represent duration of exposure in minutes. All solutions diluted to a collagen concentration of 0.4 mg/ml prior to scanning.

**Fig. 8.** UV-C degradation of collagen in the presence of OH radical scavengers or an OH radical generator. Data for 5-min exposure to UV-C of collagen solution (2 mg/ml) in 0.5 M acetic acid with different additives as follows: 100 mM thiourea (a); 100 mM cysteamine (b); no additions (c); 0.3% H₂O₂ (d). All solutions were diluted to collagen concentrations of 0.4 mg/ml prior to scanning.

**Fig. 9.** Relation between the rate constants for intermediate state formation ($k_1$) and degradation ($k_2$). Each point represents measurements of collagen solution (2 mg/ml) in 0.5 M acetic acid with different additives as follows: ■, 10 mM cysteamine; ●, 10 mM cysteamine; ▲, 100 mM cysteamine; •, no additives; ◀, 0.3% H₂O₂; ×, 100 mM cysteamine plus 0.3% H₂O₂; □, 100 mM thiourea plus 0.3% H₂O₂. All experiments were conducted at room temperature. The line represents the least squares regression line for all of the data pooled: log₁₀ $k_2$ = (1.136 ± 0.087)log₁₀ $k_1$ − 0.288 ± 0.262.
damaged triple helix was the same length as the native molecule (Table III). The intermediate state SLS crystals were less thermally stable than the native SLS crystals (Table III), reflecting the fact that the molecules themselves were intrinsically less stable, due to UV damage. The molecules in SLS crystals possessed a higher denaturation temperature than the same molecules in solution due to intermolecular interactions, as observed in fibres (14).

The formation of the intermediate state and the disappearance of the $\alpha_1$- and $\alpha_2$-chains on the gels (see Fig. 5) occurred at similar rates (compare Fig. 5 and Table II). We suggest that chain scission is predominantly random, occurring at many possible sites along the length of the chains as, for about 30 min of irradiation, the gels showed a rather uniform smearing out of the bands down the gel. However, we note that there is evidence initially of specific scission with new bands occurring down the gel. As irradiation proceeds, the number of undamaged chains declines in gels with the rate at which the intermediate state forms indicates that the critical level corresponds with at least two or all three chains being cut within the molecule.

Each scission event will cause more or less loss of thermal stability depending on its position along the chain. Our suggestion that the degradation of collagen to random coils is via the action of free radicals causing random scission along the length of all the $\alpha$-chains is consistent with the observation that the endotherms at 39 and 32 °C are caused by a population of molecules that increase in heterogeneity and fall in thermal stability as the irradiation proceeds (Fig. 4). We suggest that the effect of several chain scissions reduces $T_m$ by opening up the helix. We speculate that the intermediate state is formed once the accumulated damage destabilizes the molecule beyond a critical level, which causes the transition temperature to flip from −39 to −32 °C, i.e. to flip to the intermediate state. Since the rate constant for generating the intermediate state is faster than that for its subsequent degradation, we deduce that the average number of scissions yielding the intermediate state is less than the average number of further scissions required to reduce the intermediate state to random coils.

By drawing all of the results together, we can begin to piece together the possible sequence of events by which UV light reduces collagen molecules to random coils via an intermediate state. The primary effect of UV light is to generate free radicals in the water molecules surrounding the collagen molecule, and these radicals react with the collagen, destabilizing it. At least one of these reactions causes chain scission, which can occur at many sites along the length of the molecule, and the selection of these sites is predominantly random. The number of intact $\alpha$-chains in the population of collagen molecules therefore declines, as observed in the gels, and the number of scission points increases, as observed. While there is evidence from the gels of some new bands being formed, indicating that some sites along the $\alpha$-chains are more likely to be cut than others, the cutting is predominantly random. Thus, the majority of the $\alpha$-chain matter was smeared out along the whole length of the gel. As irradiation proceeds, the number of undamaged molecules falls and the number of damaged molecules rises. A population of new molecules with different damaged sites is produced, and these molecules have slightly different thermal stabilities, broadening the denaturation endotherm and reducing $T_{\text{max}}$. Provided the damage is less than a certain critical level, the mechanism by which the triple helix unfolds is basically unaltered, requiring the initial uncoupling of the $\alpha$-chains of the major thermally labile unit at the $C$ end of the molecule (15), followed by the rapid unzipping of the three chains along the length of the molecule. The enthalpies of activation are therefore the same in these slightly damaged molecules, and the reductions in stabilities are caused by an increase in the entropy of activation resulting from the increased flexibility of the damaged helix. Once sufficient damage has been inflicted, beyond the hypothetical critical level, the molecule becomes so unstable that the denaturation temperature flips, from its value around 39 °C to a new temperature around 32 °C. This is because the unzipping of the intermediate state needs fewer bonds to be broken initially to produce the required free energy of activation. The precise amount of damage that corresponds to the critical level has not been defined by these experiments, but comparison of the rate at which the number of undamaged chains declines in gels with the rate at which the intermediate state forms indicates that the critical level corresponds with at least two or all three chains being cut within the molecule. Thus, the single scission of a single chain is not sufficient to yield random coils, and the process of degradation of the native triple helix proceeds indirectly via an intermediate state. This intermediate state is basically triple helical and the same length as the unirradiated molecule, but with cuts in the $\alpha$-chains. It therefore has an enthalpy of denaturation very close to that of native collagen, shows a highly cooperative denaturation process, and produces SLS crystals of the same length as those of native collagen.
Further scission of the chains in the intermediate state destabilizes it even further. The $T_{\text{max}}$ of the denaturation endotherm therefore falls, while its width increases due to increasing heterogeneity in stability of the population of molecules comprising the intermediate state. Some scissions are sufficient to reduce part, or the whole, of the intermediate state molecule to random coils. With increasing irradiation times, the pool of molecules with a denaturation temperature around 39 °C declines to zero as they are reduced to the intermediate state, and all that remains are random chains and intermediate state. Finally, degradation of the intermediate state through chain scission continues until all of the molecules are reduced to random chains. The number of chain scissions required to cause complete disruption of the triple helical structure we estimate to be about 3 per chain on average, based on comparison with the gels. Since generation of the intermediate state requires on average about one cut per chain, the rate constant $k_2$ is always smaller than $k_1$.

In summary, we have shown that the collagen molecule can be quite extensively damaged by cleavage of the primary peptide bonds without disorganizing the triple helical structure. This leads to the formation of a damaged “intermediate state” prior to degradation of the molecules to random chains. The initial gelation of the solution indicates that both cross-linking and chain cleavage are occurring but that the prevalent reaction is chain cleavage by hydroxyl radicals. An understanding of the mechanisms involved will be of considerable value in future studies of the effects of UV on dermal collagen in photoaging.

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