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PAPER

Measurement of solar UV radiation in Antarctica with collagen sheets

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Collagen sheets were used in a unique evaluation method to examine skin damage caused by ultraviolet (UV) light of short wavelength during a season of the Antarctic ozone hole. The collagen sheets were exposed outdoors for 25 and 50 d, in the spring when the ozone hole was formed and in the ozone-hole-free autumn. Extracts from the exposed collagen sheets were analyzed for total protein and terminal amino acid concentrations as an index of collagen fragmentation. The results show that the amount of extractable collagen and terminal amino acid concentration in the spring exposure were approximately double and five times higher, respectively, when compared with those in the autumn exposure. During the ozone hole occurrence, the terminal amino acid concentration of the extracted collagen was about five times higher when exposure lasted 50 d from mid-September to the end of October compared to when exposure lasted 25 d from mid-September to early October. This result could be attributed to a limited amount of short-wavelength UV radiation reaching the ground surface as a result of the low height of the sun in September, when the ozone hole occurred. In fact, UV radiation measurements taken at Syowa Station indicate that short-wavelength UV radiation in the range 290–295 nm was not detected until approximately 1–2 months after the beginning of the ozone hole occurrence.

1. Introduction

The release of chlorofluorocarbons has led to ozone depletion[1–3] in the upper atmosphere of the Antarctic. Springtime Antarctic total column ozone losses (the ozone hole), first recognizable around 1980, continue to occur every year.[4] In early spring the amount of ozone decreases to one-half or one-third of its normal levels.[5,6] As ozone is depleted, the amount of ultraviolet (UV) radiation that reaches the ground surface of the Antarctic increases. The increase is most pronounced at short wavelengths. The amount of ozone tends to decrease not only in the Antarctic but also in the southern parts of Australia and New Zealand at latitude 45° S.[7] There is increased evidence that the Antarctic ozone hole has affected the surface climate in the Southern Hemisphere.[4] UV radiation with wavelengths below 320 nm severely affects life, including humans, as it can cause skin problems from mild disorders to solar keratosis, which has a risk of progressing to skin cancer.[8]

It is essential to study human skin damage caused by short-wavelength UV radiation during ozone hole occurrence. However, the dangers involved mean that it is difficult to evaluate the damage by simple and quantitative methods. It has been known that UV degrades collagen molecules.[9] There is also a report that, upon UV radiation, skin cells secret a degrading enzyme to degrade collagen.[10] The present authors have therefore proposed a unique evaluation method in which collagen sheets are used instead of human skin.[11] In a previous study, collagen sheets were laminated with various UV protection films and exposed outdoors in a preliminary experiment in the Antarctic in summer to examine the protective effect of the UV protection films on the collagen sheets.[12] In this study, an outdoor exposure test was conducted in the Antarctic in spring (September–October), when the ozone hole occurred. For comparison, an outdoor exposure test was also performed in autumn (February–March), when the height of the sun is exactly the same. The results in both seasons were compared to study the effect of UV radiation on the skin during the ozone hole occurrence.

2. Experimental

2.1 Materials

2.1.1 Collagen sheets. Collagen fiber from pork skin was dispersed in distilled water, and the dispersion was adjusted to pH 3.5 with citric acid. After homogenization with a Waring blender, the collagen dispersion was de-foamed and adjusted to a final concentration of 2.0 wt%. A freeze-dryer was used to freeze-dry the collagen dispersion. The sponge-like structure formed was sliced to a thickness of 1.5 mm to obtain collagen

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sheets with a unit area weight of 29 g m^{-2}.[11] The collagen sheets measured 1500 μm in thickness under a pressure of 0 gf cm^{-2}, 1402 μm under 50 gf cm^{-2}, and 1193 μm under 240 gf cm^{-2}. Collagen fibers from the pork skin were used in this experiment because porcine skin is believed to be similar to human skin.[13]

2.2 Method of exposure

2.2.1 Exposed samples. The collagen sheets were laminated in five layers for outdoor exposure in the Antarctic (Fig. 1). To avoid the influence of winds and snow, the collagen sheets were covered with a polyethylene film 12.16 μm in thickness (Fig. 1). This extremely thin film has a very high transmittance for UV radiation. The polyethylene film used in this study transmits 88% of UV radiation at 280 nm, 92% of that at 310 nm, and 89% of that at 350 nm, which show high values. Polyethylene is a material that tolerates low temperature and does not degrade readily when exposed to UV radiation.

2.2.2 Exposure unit. The 48th Japanese Antarctic Research Expedition set up an exposure stand at Syowa Station on East Ongul Island, which is located along the coast of the Lutzow-Holm Bay in the Antarctic. Fig. 2 shows a map of the area around Syowa Station. The exposure site is at latitude 69°00′ S. and longitude 39°35′ E. Fig. 1 is a photograph of the exposure stand taken from the north side. Samples for exposure were placed in the vertical direction (facing up) or in the horizontal direction (facing true north) on the 1.5 m high exposure stand. Samples that are facing up are mounted on a horizontal plate, while samples facing north are mounted to a vertical board. The samples (10 × 12 cm) were fixed to the exposure stand at intervals to prevent them overlapping. The present paper reports only the results obtained from exposure in the vertical direction (facing up) because few differences were found between samples exposed in the vertical direction (facing up) and those exposed in the horizontal direction (facing true north).

2.2.3 Duration of exposure. Fig. 3 shows the changes in the total ozone column at Syowa Station in the four years prior to the experiment (2003–2006).[14] The amount of ozone varied by year but substantially decreased below 220 m atm-cm during

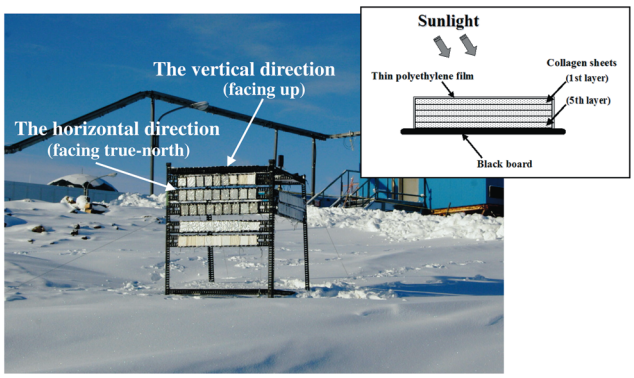

Fig. 1 Sunlight exposure experiments on the north side for collagen sheets at Syowa Station, Antarctica. A schematic representation of sunlight exposure samples.

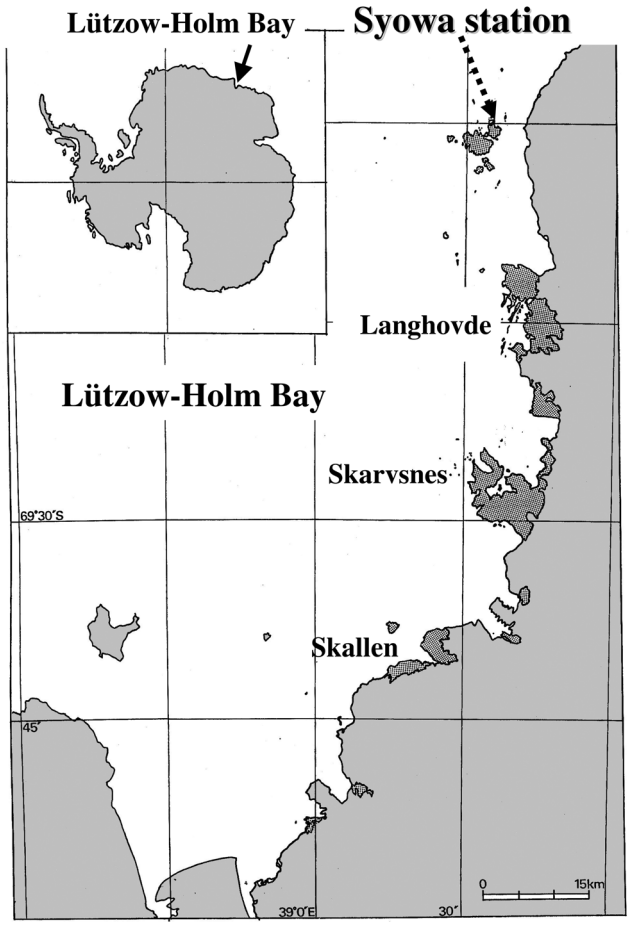

Fig. 2 A map of the area surrounding Syowa Station, East Antarctica.

September to October, in the spring. This confirmed the appearance of the so-called ozone hole. Therefore, the 25 d from 11 September to 5 October of 2007 and 50 d from 11 September to 30 October were set as exposure periods in the spring, when the ozone hole is known to occur. For comparison, the collagen sheets were also exposed for 25 d from 9 March to 2 April and for 50 d from 12 February to 2 April in the autumn of 2007. Mid-winter in 2007 was 22 June. In other words, the spring and autumn exposures in this experiment were set so that the sun was at the same height during both periods.

As explained earlier, it is in spring that the ozone hole appears to allow short-wavelength UV radiation to reach the ground surface, and in autumn no ozone hole appears. In short, a comparison of exposure results in spring and in autumn allows a quantitative examination to be made of the effect of the presence of high-energy UV radiation. To evaluate the effect of UV radiation alone, collagen sheets covered with aluminum foil to intercept sunlight were also fixed to the exposure stand for comparison.

2.3 Measurements

2.3.1 Observation under a scanning electron microscope. A carbon tape for vapor deposition was pasted on a stage for electron microscopy, and a sample was fixed onto the tape. After

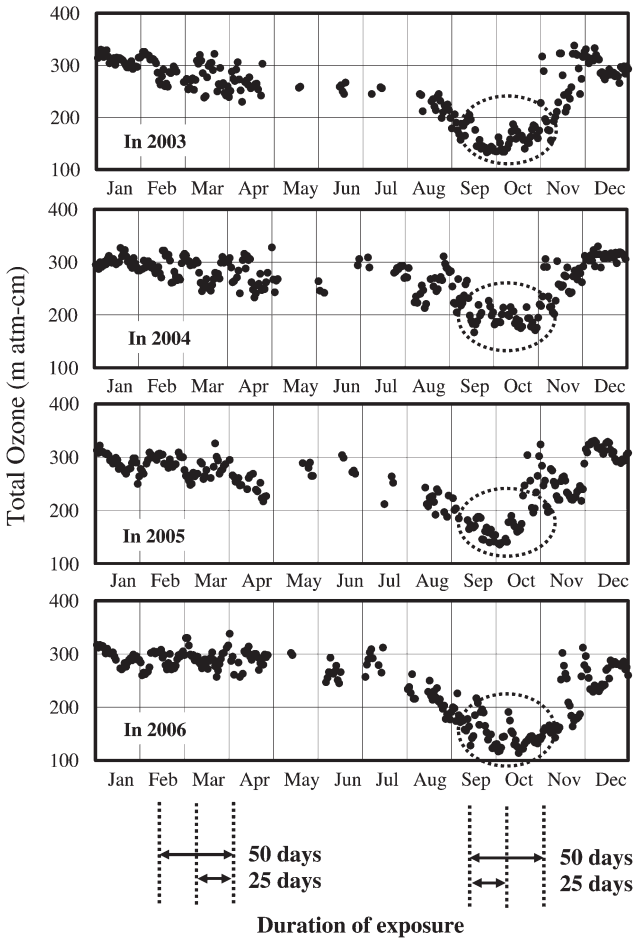

Fig. 3 Total amount of ozone and annual variations in daily representative data at Syowa Station in 2003–2006. The circular dotted lines indicate formation of the ozone hole. The ozone column is the height of a hypothetical layer of pure ozone which would result if all ozone molecules in a vertical column above the Earth's surface were brought to standard pressure (1 atm) and standard temperature (273.15 K). 1 m atm-cm corresponds to a height of 0.01 mm of this hypothetical layer.

drying the fixed sample at room temperature for 24 h, gold was vapor-deposited on the sample using an ion sputter E-1010, Hitachi High-Technologies Corporation. Then, an S-3000N scanning electron microscope (SEM), Hitachi Science Systems Co., Ltd, was used to observe the surface of the exposed collagen sheets. SEM was performed at an accelerating voltage of 20 kV.

2.3.2 Changes in color of exposed collagen sheets. To study changes in the color of the exposed collagen sheets, light reflected by the exposed collagen sheets was measured for reflectance using a UV-3100 spectrophotometer, Shimadzu Corporation, to which the integrating sphere was attached. Spectra for a wavelength range of 220–700 nm were obtained under the conditions of 2.0 nm slit-width and 0.5 nm sampling-pitch. Barium sulfate powder was caked to prepare a plate, which was used as a standard white plate.

2.3.3 Preparation of extracts from collagen sheets. It was decided to extract collagen in order to analyze the collagen sheets. A sheet of the exposed collagen was fine cut to a size of 3 × 3 mm, and about 0.1 g of the cut sheet was transferred to a weighing bottle and immersed in 100 times the amount of a 50 mM aqueous acetic acid solution. A shaker was used to shake the bottle at a speed of 120 rpm for 24 h. The supernatant was collected to obtain a collagen extract.

2.3.4 Amino acid analysis and burette reaction. An equivalent amount of 12 N HCl was added to 100 μL of a collagen extract obtained as described in Section 2.3.3 for hydrolysis at 110 °C for 24 h. Hydrochloric acid was removed by evaporation to dryness after the reaction. The residue was re-dissolved in 150 μL of 0.02 N HCl to obtain a sample for amino acid analysis. An L-8800 high-speed amino acid analyzer, Hitachi High-Technologies Corporation, was used to calculate the total amount of detected amino acids and the amount of hydroxyproline.

A mixture of 42 mL of a 1.83×10^{-2} mol L^{-1} aqueous copper (II) sulfate solution and 80 mL of a 9.49 mol L^{-1} aqueous sodium hydroxide solution was stirred to prepare a burette reagent, 500 μL of which were added to 1000 μL of the collagen extract. The mixture was stirred and left standing for 10 min. A spectrophotometer was used to measure the absorbance at 310 nm to calculate the total protein content of the collagen extract. Different concentrations of collagen standard solutions (0.01–0.10%) were used to quantify total protein.

2.3.5 Quantification of terminal amino group concentration of extracts. To a test tube containing 0.1 mL of a collagen extract obtained as described in Section 2.3.3, 1 mL of a ninhydrin solution (propyleneglycol monomethyl ether, ninhydrin), Wako Pure Chemical Industries Co., Ltd, and 1 mL of a buffer solution (propyleneglycol monomethyl ether, lithium acetate dihydrate), Wako Pure Chemical Industries Co., Ltd, were added. The mixture was heated at 100 °C for 10 min and left standing at room temperature for about 3 h. A spectrophotometer was used to measure sample solutions for absorbance at 570 nm.

A molecule of bovine albumin has one terminal amino group, as do collagen molecule chains. Different concentrations of bovine albumin solutions were prepared and treated in the same way as the collagen extract to measure absorbance. Assuming that ninhydrin reacts only with the terminal amino groups, a working curve was prepared for terminal amino group concentration and absorbance. Terminal amino group concentrations of the test solutions were calculated from the absorbance of the test solutions.

2.3.6 Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). A Mini-Protean 3 cell, Bio-Rad Japan Co., Ltd, was used for electrophoresis. First, a freeze-dryer FD-1000, Tokyo Rikakikai Co., Ltd, was used to freeze-dry and concentrate 0.1 mL of collagen extracts. Then, a 5% stacking gel 0.75 mm in thickness and a 15% separation gel were prepared using the method described by Laemmli.[15,16] To a concentrated collagen extract, 15 μL of a Laemmli sample buffer containing 5% 2-mercaptoethanol were added, and the mixture was heated at 95 °C for five min. The whole test sample solution was injected into a well of the gel, and electrophoresis was performed at a constant voltage of 100 V for about 2 h. Bands were stained with Coomassie Brilliant Blue R-250 for detection. As molecular-weight markers, 10 μL of Precision Plus Protein™ All Blue Standards was used.

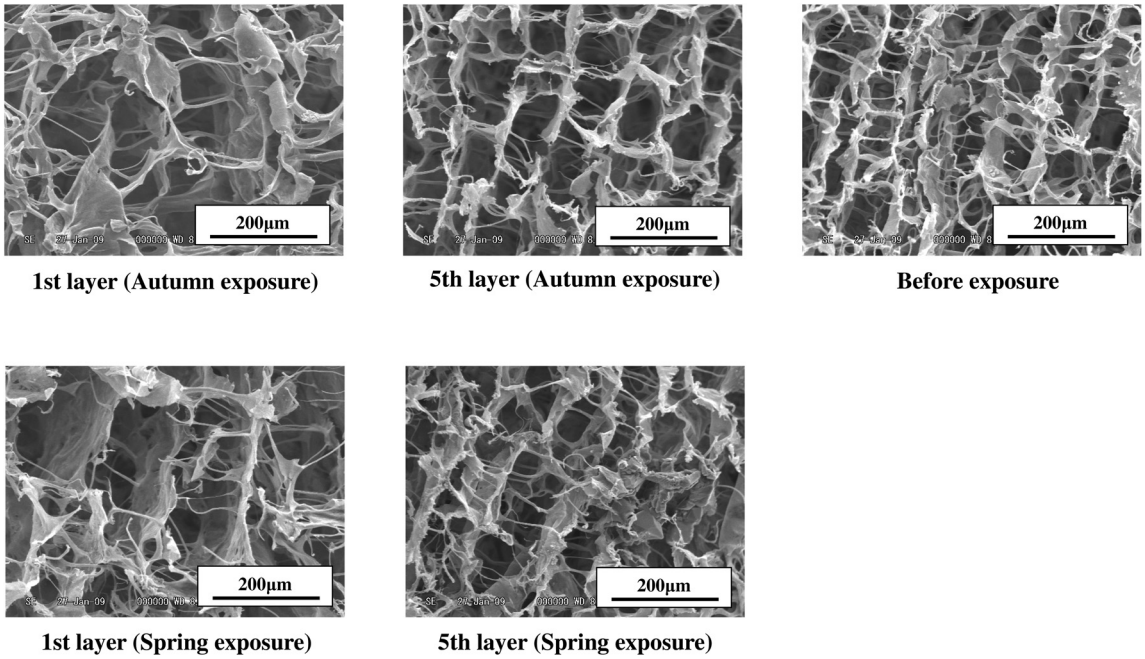

Fig. 4 Scanning electron micrographs of layered collagen sheets after sunlight exposure in the vertical direction (×200).

3. Results and discussion

3.1 Morphological observation of exposed collagen sheets under electron microscope

The collagen sheets were laminated in five layers (Fig. 1) and exposed outdoors in the autumn, when the ozone hole did not occur, and in the spring, when the ozone hole did occur. The durations of exposure were 25 d and 50 d in both seasons. To examine the effect of short-wavelength UV radiation on the collagen sheets, an SEM was used to make morphological observations of the exposed collagen sheets for 50 d (Fig. 4). The materials observed were the outermost layers (the first layers) and the fifth layers of collagen sheets that were laminated and exposed as described in Section 2.2.1. Materials exposed in the vertical direction (facing up) in the spring, when the ozone hole occurred, were compared with those exposed in the same direction in the autumn, when the ozone hole did not occur.

The results show that the outermost layers (the first layers) of the collagen sheets had cleavages in the sponge-like structure, or collapsed partly, in both the spring and the autumn exposures. The observation showed little structural difference between the spring and autumn exposures. The material exposed in the spring turned more yellowish. In contrast, the fifth layers of the collagen sheets had a sponge-like structure with orderly, fine hollows in both the spring and autumn exposures. Few differences were found between the two structures and between the two structures and those of the collagen sheets before exposure. In other words, UV radiation was found to exert no effect on the structure of the fifth layers of the collagen sheets.

To find quantitative changes in the hue of the exposed first layers of the collagen sheets, a spectrophotometer was used to measure the reflectance spectra of the reflected light. Fig. 5 shows the results of 25 d and 50 d exposure in the vertical direction (facing up). The 50 d exposures had lower reflectances than the 25 d exposures in both the spring and the autumn exposures, revealing more browning. The results indicate that 50 d exposures in the spring had lower reflectance than the other exposure durations, revealing the most browning. From these results, it is believed that short-wavelength UV radiation caused the collagen molecule chains to undergo chemical and color changes in the 50 d spring exposure.

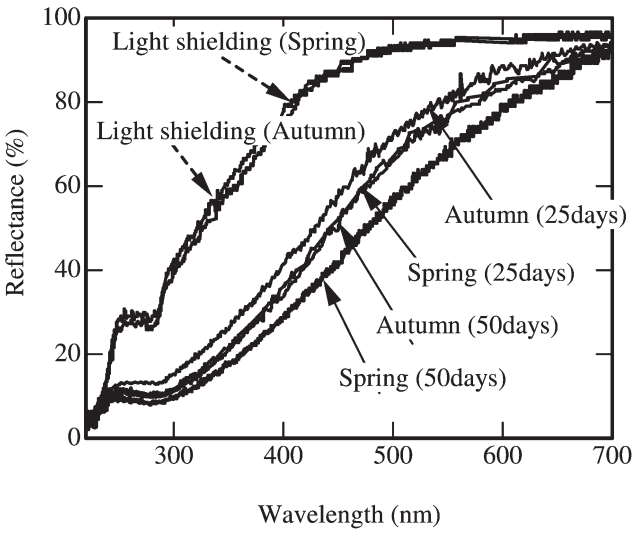

Fig. 5 The reflectance of layered collagen sheets after sunlight exposure for various periods of vertical exposure (facing up).

In contrast, the shielded collagen sheets exhibited no difference between the spring and the autumn exposures and relatively high reflectance. An evaluation by visual inspection also

Table 1 Amino acid amounts for extracts from layered collagen artificial skins after sunlight exposure for 50 d of vertical exposure (facing up)

	Amounts of amino acids (μg (100 μl)$^{-1}$)	1st layer	2nd layer	3rd layer	4th layer	5th layer	Light shielding
Autumn exposure	Total	196.8	89.9	53.7	29.8	22.4	11.1
	Hydroxyproline	22.49	10.30	7.02	3.32	2.34	1.15
Spring exposure	Total	249.4	110.7	109.9	76.9	37.4	10.4
	Hydroxyproline	30.99	13.70	13.68	9.38	4.47	1.01

indicated little difference between the shielded collagen sheets and the collagen sheets before exposure.

As explained above, spectrophotometric measurements also quantitatively confirmed color changes. UV radiation seemed to cause chemical changes in the collagen sheets and render them fragile.

3.2 Effects of short-wavelength UV radiation on collagen sheets

Collagen fiber hardly dissolves in water, unless it is damaged. Therefore, extracts were prepared as described in Sections 2.3.3 and 2.3.4 from the collagen sheets that were exposed in the vertical direction (facing up) for 50 d and analyzed for amino acids. Table 1 lists the total amounts of detected amino acids and the amounts of detected hydroxyproline, which is intrinsic to collagen. Both the total amount of amino acids and the amount of hydroxyproline were largest in the first, outermost layers, and decreased from the second layers to the fifth layers. In other words, UV radiation was shown to break more collagen molecules and to render collagen molecules more readily extractable with acetic acid in the layers closer to the outer surface. The spring exposure produced larger amounts of both compared to the autumn exposure. This is believed to be caused by short-wavelength UV radiation that broke collagen molecule chains and rendered them readily extractable with acetic acid.

Data on the amino acid analysis of extracts show that glycine comprises about one-third of the composition, and that the ratios of proline, hydroxyproline, and alanine were comparable with those of collagen fiber. This indicates that the extracted components were fragments from the collagen.

Extracts were then prepared from the collagen sheets exposed in the vertical direction (facing up) and examined for total protein and terminal amino group concentration. The method for determining "total protein" is described in Section 2.3.4 and that for determining "terminal amino group concentrations" are described in Section 2.3.5. Fig. 6 gives the results of 25 d and 50 d exposures in the autumn and in the spring. The total protein is the amount of components that are rendered readily extractable with acetic acid by exposure of the collagen sheets to UV radiation and is proportional to the amount of low molecular-weight substances produced by UV radiation. In contrast, terminal amino group concentration is proportional to the amount of collagen molecule chains that are broken by UV radiation. Total protein and terminal amino group concentration of the collagen extracts were largest in the first, outermost layers, in both the 25 d and the 50 d exposures. These values were confirmed to decrease from the second layers to the fifth layers. In short, the data quantitatively show that the effect of UV radiation was greater in the layers closer to the outer surface of the laminated collagen sheets.

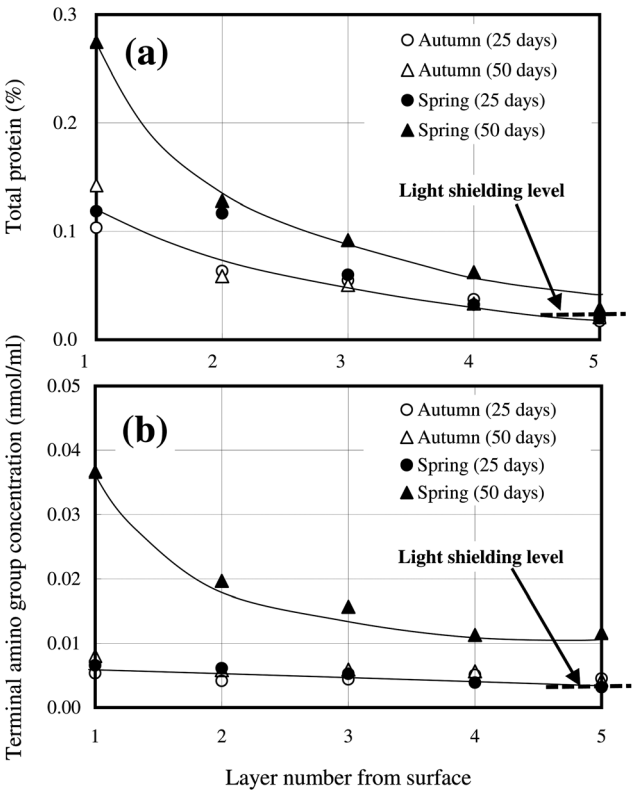

Fig. 6 Structural analysis of extracts from layered collagen sheets after sunlight exposure: (a) total protein, and (b) terminal amino group concentrations.

Collagen degradation was found to be particularly great in the 50 d spring exposure compared with the 25 d spring exposure and 25 d and 50 d autumn exposures. It should be noted that, although total protein was about twice as much in the 50 d spring exposure as in the 50 d autumn exposure, terminal amino group concentration in the former was about five times as high. This clearly indicates that exposure to short-wavelength UV radiation broke collagen molecule chains into finer pieces in the spring. In other words, the effect of the ozone hole is evident. It should also be noted that terminal amino group concentration was about five times as high in the 50 d exposure as in the 25 d exposure in the spring, when the ozone hole occurred. The reason for this will be explained later.

For comparison, the collagen sheets were shielded with aluminum foil and exposed on the exposure stand for 50 d, both in the spring and in the autumn. The broken lines in Fig. 6 indicate the results. There was little difference between the autumn exposure and the spring exposure. Furthermore, total protein and terminal amino group concentration were nearly the same in the collagen

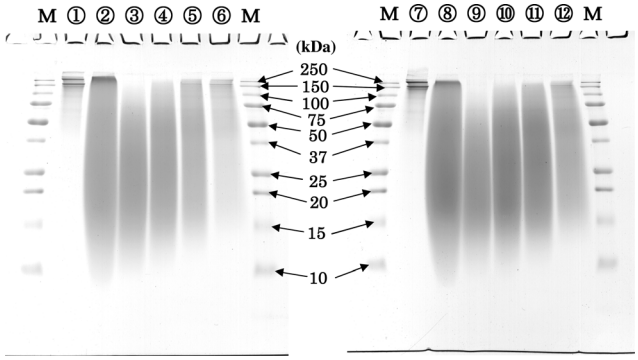

Fig. 7 SDS–PAGE of extracts from layered collagen sheets after sunlight exposure for 50 d of vertical exposure (facing up): left: autumn exposure, and right: spring exposure. ① Light shielding (autumn), ⑦ light shielding (spring), ②⑧ 1st layer, ③⑨ 2nd layer, ④⑩ 3rd layer, ⑤⑪ 4th layer, and ⑥⑫ 5th layer; M: molecular marker.

sheets shielded with aluminum foil and in the exposed fifth layers. In short, UV radiation exerted little effect on total protein and terminal amino group concentration in the fifth layers. These values were also nearly the same as those for the collagen sheets before exposure. From these results, it may be stated that the degradation of the exposed collagen sheets were caused by UV radiation alone and that factors other than UV radiation, such as temperature and humidity, exerted no effect.

Thus, the extent of degradation caused by UV radiation differed markedly between the spring, when the ozone hole occurred, and the autumn, when the ozone hole did not occur. Even during the 50 d spring exposure, when the ozone hole occurred, the effect of UV radiation was found to vary greatly between the earlier period of 11 September to 5 October and the later period of 6–30 October.

Fig. 7 shows the results of electrophoresis of the collagen sheets exposed for 50 d in the vertical direction (facing up). The left-hand gels in Fig. 7 indicate the results of exposure in the autumn, and the right-hand gels indicate the results of exposure in the spring. Also shown are the results of the shielded collagen sheets set up in each of the exposure seasons. When the collagen sheets were shielded, bands of collagen molecule chains, α-chains and β-chains, appeared in both exposure seasons. In contrast, all of the bands disappeared in the exposed collagen sheets.

In the first, outermost layers, many degradation products from the collagen molecule chains, which were as low in molecular weight as 15 kDa, were observed. A comparison of the exposure result in the spring with that in the autumn clearly showed more substances of low molecular-weight in the spring exposure. High-energy UV radiation produced more degradation products of low molecular-weight from collagen molecule chains. This appears to be the result of ozone hole occurrence in the spring. This is clearly the effect of short-wavelength UV radiation. Products of low molecular-weight, about 15 kDa, generated by high-energy UV radiation, were fragments from collagen and formed through collagen degradation. As explained above, it was found that UV radiation not only formed bridges in collagen molecule chains in the collagen sheets but also degraded the collagen molecule chains.

3.3 Total column ozone and UV radiation in the upper atmosphere of the Antarctica

As explained above, the effect of UV radiation on the collagen sheets varied greatly between 11 September and 5 October (25 d) and 11 September and 30 October (50 d) in the spring exposure, when the ozone hole is known to occur. Therefore, attention was paid to determining the relation between the total column ozone and UV radiation. Fig. 8 shows daily average values of the total column ozone and the daily UV dose at the surface above Syowa Station in 2007, measured by the Japan Meteorological Agency.[17] Fig. 8(a) shows the total column ozone and Fig. 8(b) indicates the UV radiation. A Dobson Ozone spectrophotometer was used to measure total column ozone and a Brewer spectrophotometer was used to measure UV radiation.

The data show that the total column ozone during the exposure period of 11 September to 30 October in the spring was below 220 m atm-cm, which is the defined limit of the ozone hole. In short, the ozone hole did occur in 2007. The total column ozone was found to recover considerably to 230–330 m atm-cm in November. The total column ozone is usually in the range 250–450 m atm-cm around Japan.

The UV radiation that reached the ground surface is presented as a curve with the minimum value near 22 June, which was

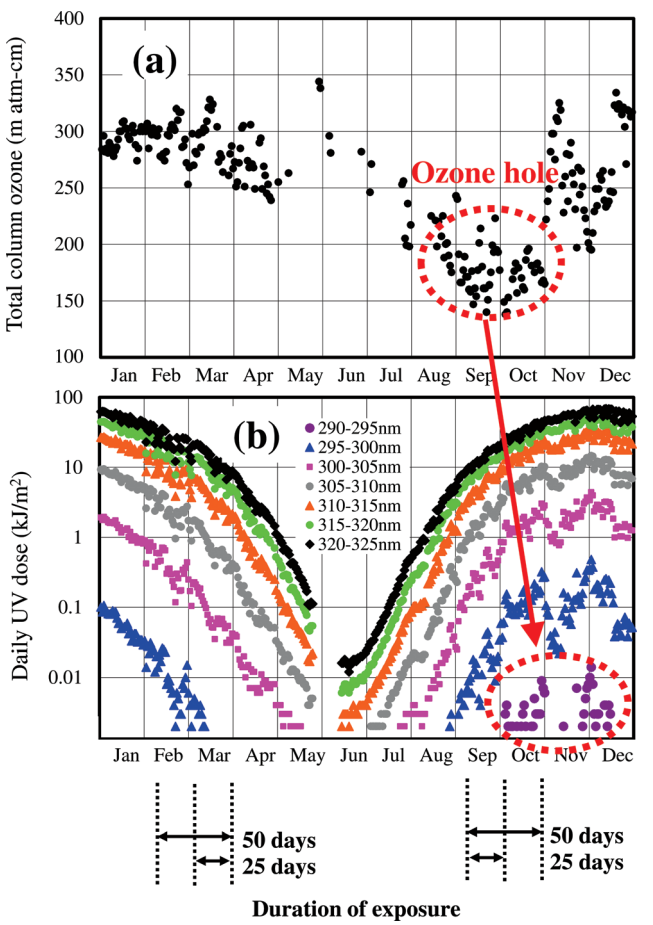

Fig. 8 Annual variations in daily representative data at Syowa Station in 2007: (a) total column ozone, and (b) daily UV dose.

mid-winter (Fig. 8b). It should be noted here that short-wavelength UV radiation in the range 290–295 nm was measured during October–December. It should also be noted that short-wavelength UV radiation did not reach the ground surface in September (Fig. 8b), when the ozone hole had already occurred (Fig. 8a). Short-wavelength UV radiation reached the ground surface even during November–December, when the total column ozone had already recovered (Fig. 8b).

Surface UV radiation depends mainly on the solar elevation, the total ozone column, and clouds. Solar elevation from the horizontal line was in a range of 15–26 degrees even at noon during the 25 day exposure (11 September to 5 October). UV levels remained small despite the low total ozone column during this period. However, during the 25 d spring exposure period (11 September to 5 October) of this study, UV radiation from the sun was low because of the low height of the sun. In contrast, the sun rose higher during the later period of the 50 d spring exposure (5–30 October), increasing UV radiation. In other words, although the amount of short-wavelength UV radiation (290–295 nm) that reached the ground surface was relatively small during 11 September to 5 October because UV radiation from the sun was low, it is believed that solar radiation increased, thus increasing the amount of UV radiation (290–295 nm) that reached the ground surface during 5–30 October, when the total column ozone in the upper sky of the Antarctic was little different from that during 11 September to 5 October.

Commission Internationale de l'Eclairage has defined the CIE action spectrum for relative levels of UV effects on humans by wavelength.[18] This CIE action spectrum shows that UV of short wavelengths below 300 nm exerts great effects on humans.[18] According to the report,[19] UV of shorter wavelengths causes greater damage on collagen. In other words, larger amounts of short wavelength UV radiation are believed to damage collagen to greater extents in exposure in Antarctica during ozone hole occurrence. The present study revealed that the 50 day exposure greatly damaged collagen in the spring when UV radiation in a wavelength range of 290–295 nm reached the ground. This is very interesting in that the damage of the collagen sheets exposed in the spring is associated with ozone hole occurrence.

As explained above, short-wavelength UV radiation reached the ground surface 1–2 months later than when the total column ozone began to decrease in the upper sky of the Antarctic. Because of this, the amount of short-wavelength UV radiation that reached the ground surface varied greatly between the 25 d period from mid-September to early October and the 50 d period from mid-September to the end of October during the exposure when the ozone hole occurred. The experiments in this study with the collagen sheets also confirm that short-wavelength UV radiation does not necessarily reach the ground surface when the ozone hole occurs but reaches the ground surface 1–2 months after the ozone hole begins to occur.

4. Conclusions

Collagen sheets were used in a unique evaluation method to study the effects of UV radiation on human skin during a period of the Antarctic ozone hole.

(1) Collagen sheets were laminated and exposed outdoors. Terminal amino group concentrations in the extracts from the collagen sheets exposed in the spring were found to be about five times as high as from those exposed in the autumn.

(2) There was a five-fold difference in the terminal amino group concentration of the collagen extract between the period from mid-September to early October (25 d) and the period from mid-September to the end of October (50 d) during the spring exposure when the ozone hole occurred. In other words, the effect of short-wavelength UV radiation was much greater when exposed in October than in September.

(3) The total column ozone above Syowa Station decreased to below 220 m atm-cm during September–October. The data confirm ozone hole occurrence. During November–December, the total column ozone recovered to about 230–330 m atm-cm. On the other hand, little short-wavelength UV radiation (290–295 nm) reached the ground surface in September, when the ozone hole occurred, but began to reach the ground surface in October–December. In other words, short-wavelength UV radiation was found to begin to reach the ground surface approximately 1–2 months after the start of ozone hole occurrence.

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