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Using collagen artificial skin to estimate the protection effects of UV-cut materials against sunlight under the Antarctic ozone hole

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ABSTRACT

Collagen sheets that simulate human skin were employed to study the protection effects of ultraviolet-cut (UV-cut) films on the skin when the Antarctic ozone hole appeared. A collagen sheet was covered with a polypropylene film containing zinc oxide and exposed outdoors in the Antarctic. Exposed sheets were dissolved to determine total amino acid amounts. The results show that nearly 2.8 times as much total amino acids were produced in collagen sheets exposed in spring, when the ozone hole appeared, as those produced in collagen sheets exposed in autumn. However, total amino acids in a collagen sheet covered by a film with a zinc oxide content of 0.40 v% decreased to nearly one-fourth the amount in a collagen sheet covered with a zinc-free film, even during spring exposure. Furthermore, analysis shows that total protein and terminal amino group concentration decreased substantially in extracts from collagen sheets with increasing levels of zinc oxide in the film. In other words, the addition of zinc oxide is confirmed to suppress collagen deterioration by UV light very effectively, even if exposure lasts 50 d in spring, when the ozone hole appears. As described above, the collagen sheet method used for evaluation could be used to quantify the protection effects of UV-cut film against high-energy UV light that reaches the ground when the ozone hole appears.

1. Introduction

Environmental destruction has brought about the ozone hole [1–3] in the upper sky of Antarctica and pouring short-wavelength UV on the ground [4,5]. Consequently, there are intense concerns about UV effects on biopolymer in the skin and eye. Short-wavelength UV acts on collagen, extracellular matrix [6], in the skin to cause dermal damage, such as wrinkles, slack, and blemishes [7,8]. It is important to study effects of short-wavelength UV on the cross-linking and degradation of collagen, a biopolymer, during ozone hole occurrence. However, the effects are difficult to estimate safely and quantitatively.

Many UV-cut products, such as sunscreens and face masks, have also been developed to protect the skin from UV. However, it is also difficult to examine their protection effects against pouring short-wavelength UV during ozone hole occurrence. Therefore, collagen sheets [9,10] were prepared from porcine dermis and covered with UV-cut films to be used in exposure experiments in Antarctica during ozone hole occurrence [11]. The purposes are to examine the effect of short-wavelength UV on the degradation of collagen, a polymer, and those of UV-cut films on collagen stability.

2. Experimental

2.1. Materials

2.1.1. Collagen sheets

Collagen fiber from porcine 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 sheets (artificial skin) with a unit area weight of 29 g/m^2 [9]. The collagen sheets measured 1500 μm in thickness under a pressure of 0 gf/cm^2, 1402 μm under 50 gf/cm^2, and

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1193 μm under 240 gf/cm^2. Collagen fibers from porcine skin were used in this experiment because porcine skin is believed to be similar to human skin. These sponge-like collagen sheets are referred to as "collagen sheets" hereafter [9].

2.1.2. UV-cut film

Zinc oxide (molecular weight: 81.39), a UV-scattering agent, 25 nm in particle size was added to polypropylene (molecular weight: 3.0×10^5) to prepare films of thickness 50 ± 2 μm with a T-die film machine [9]. This UV-scattering agent is an inorganic solid that physically reflects and scatters UV light to provide UV protection. Fig. 1(b) shows the absorbance spectra of the films. The figure indicates that the added zinc oxide provides effective protection from UV light with wavelengths shorter than 370 nm. Amounts added are expressed in volume percent (v%).

2.2. Outdoor exposure in the Antarctic

2.2.1. Method of exposure

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). A collagen sheet was covered with a UV-cut film and pasted on an exposure stand to be exposed outdoors (Fig. 1(a)). The exposure site is at latitude 69°00′ S and longitude 39°35′ E (Fig. 2). 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. Fig. 3 is a photograph of the exposure stand taken from the north side. Directions mentioned here were true directions, not magnetic directions. The samples (10×12 cm) were fixed to the exposure stand at intervals to prevent them overlapping.

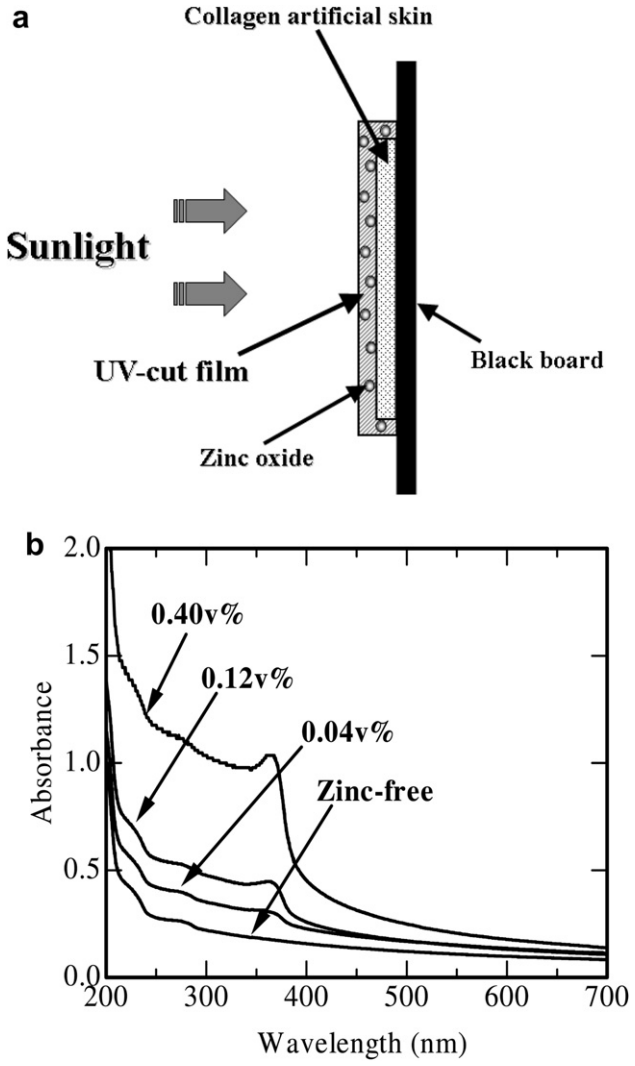

Fig. 1. Schematic representation of sunlight exposure samples and absorbance spectra of UV-cut films: (a) schematic representation of artificial skin (collagen sheets) covered with UV-cut film containing zinc oxide, and (b) relationship between wavelength and absorbance of UV-cut polypropylene films for various zinc oxide contents.

2.2.2. Duration of exposure

The ozone hole is known to appear in the Antarctic from September to October in the spring. Therefore, 25 d from 11 September to 5 October 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, of 2007. 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. The reasons for carrying out this exposure experiment in these seasons are explained in Section 3.1. Mid-winter in 2007 was 22 June. In other words, the spring and autumn exposures in this experiment were set in the periods when the sun was at the same height during both periods.

As explained earlier, it is in spring when the ozone hole appears to allow short-wavelength UV light to reach the ground surface, and in the 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 short-wavelength UV light. To evaluate the effect of UV light 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. Absorbance spectra of films

Transmitted light through the films used in the experiment was measured for absorbance with a recording spectrophotometer UV-3100, Shimadzu Co., Ltd., to which an integrating sphere was attached. Absorbance spectra for a wavelength range of 200–700 nm were obtained under the conditions of 2.0-nm slit-width and 0.5-nm sampling-pitch.

2.3.2. Changes in the color of exposed collagen sheets

To study changes in the color of the exposed collagen sheets, light reflected by the sheets was measured for absorbance with the spectrophotometer. Absorbance spectra for a wavelength range of 220–700 nm were obtained under the conditions of 2.0 nm in slit-width and 0.5 nm in sampling-pitch. An absorbance value of 0 was used for a standard white plate to achieve calibration.

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 were 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 alone to obtain a collagen extract.

2.3.4. Amino acid analysis and Biuret 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

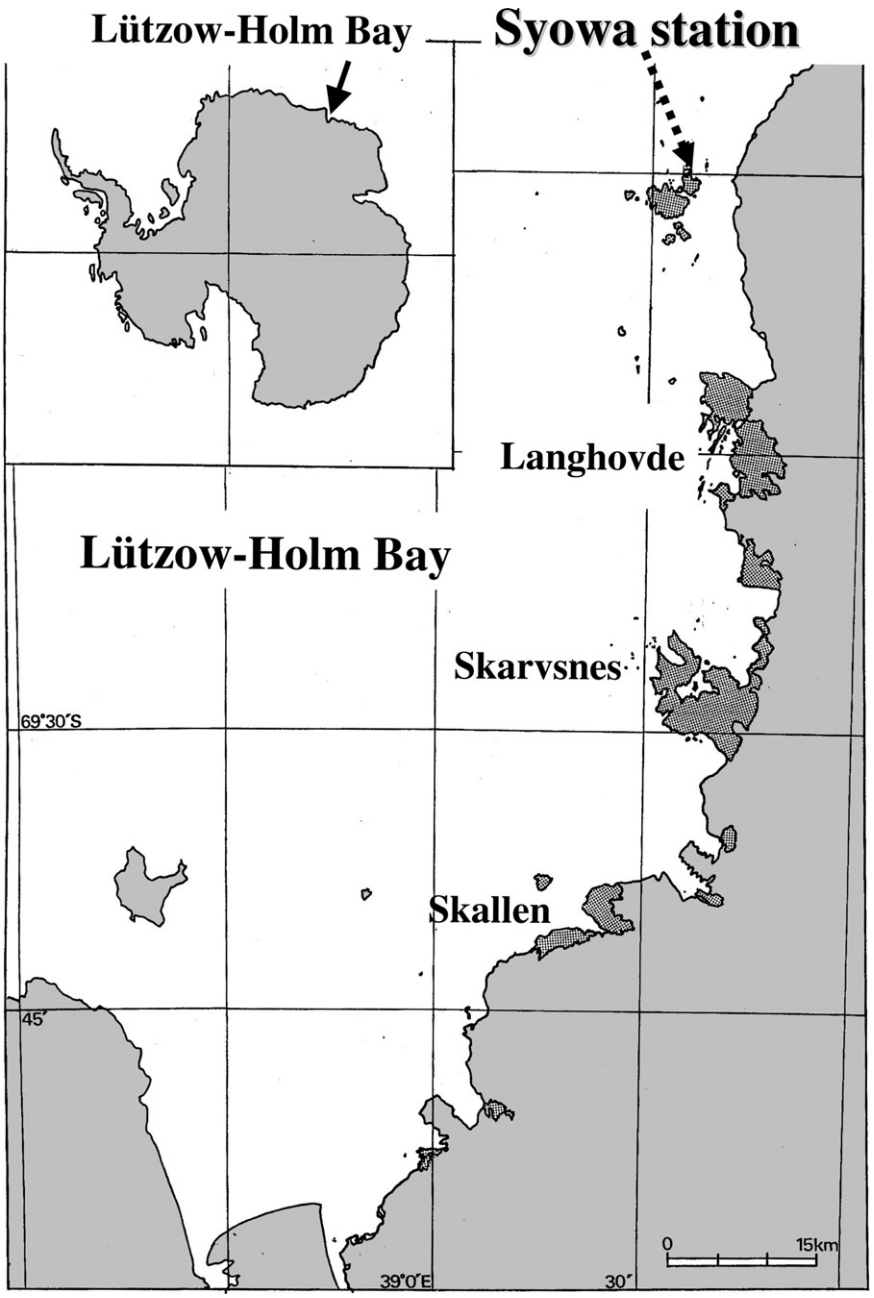

Fig. 2. Map of area surrounding Syowa Station, East Antarctica.

evaporation to dryness after the reaction. The residue was redissolved 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 for amino acid analysis 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 aqueous copper(II) sulfate solution and 80 mL of a 9.49 mol/L 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. The spectrophotometer was used to measure the absorbance at 310 nm to calculate the total protein of the collagen extract. Various concentrations of collagen standard solutions (0.01–0.10%) were used to quantify total protein.

2.3.5. Quantification of terminal amino group concentrations 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. The 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. Various concentrations of bovine albumin solutions were prepared and treated in the same way as

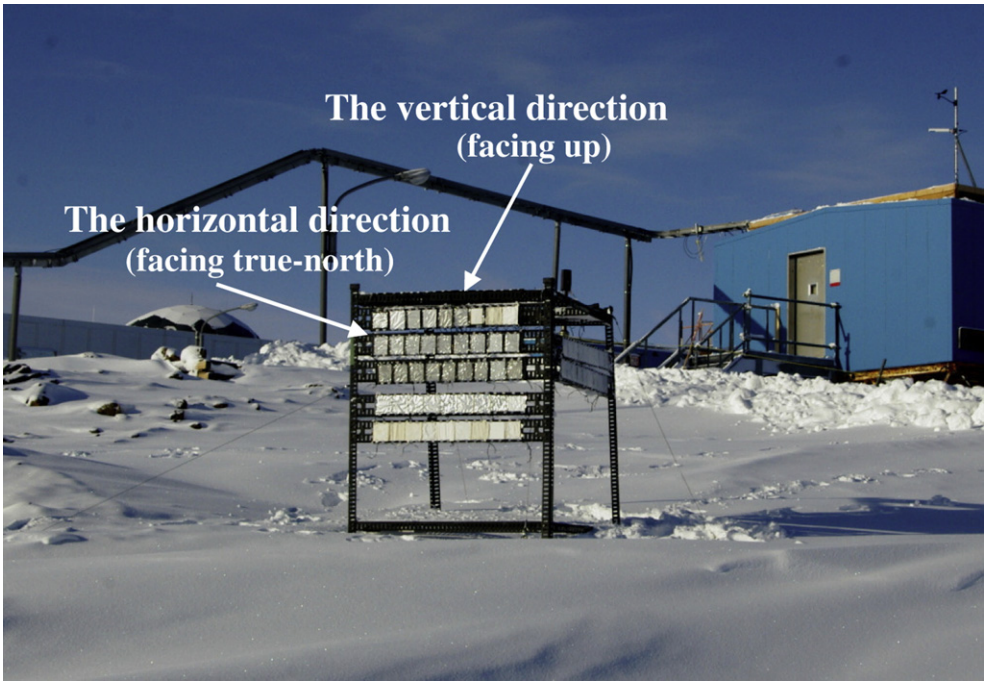

Fig. 3. Sunlight exposure experiments on the north side for collagen sheets at Syowa Station, Antarctica.

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 absorbances 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 [12,13]. 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 5 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.

3. Results and discussion

3.1. Relations between UV radiation and total amount of ozone in the atmosphere above Syowa Station

Temperatures in the sky above Syowa Station rise with increasing air temperature for six months from winter to summer. A survey was therefore conducted on stratospheric temperature above Syowa Station and on relations between the total amount of ozone in the stratosphere and UV radiation reaching the ground during the exposure period from June to December 2007 [14]. Fig. 4(a) shows how temperature at 100 hPa relates to the total amount of ozone in the sky above Syowa Station, and Fig. 4(b) indicates how this temperature relates to UV radiation of each wavelength reaching the ground. The position at 100 hPa in the sky corresponds to the lower stratosphere, i.e., about 14.5–19.0 km in altitude.

As Fig. 4(a) shows, there were periods during which the total amount of ozone in the sky above Syowa Station was under 220 m atm-cm, which is the line of demarcation for the definition of an ozone hole. In short, the ozone hole did appear in 2007. Moreover, as the figure shows, the total amount of ozone increased to a range of 230–340 m atm-cm as the temperature rose above −60 °C, and the ozone layer recovered substantially. The total amount of ozone is usually in the range 250–450 m atm-cm around Japan.

In contrast, UV radiation of every wavelength reaching the ground can be described as a curve extending upward with a maximum value in the stratospheric temperature range from −65 °C to −60 °C (Fig. 4(b)). It should be noted that UV radiation of 290–295 nm was measured when the stratospheric temperature was in the range −75 °C to −55 °C. As Fig. 4(a) indicates, the ozone hole disappeared when the temperature was above −60 °C. In other words, short-wavelength UV light of 290–295 nm reached the ground when the stratospheric temperature was −65 °C to −55 °C, although the ozone hole had disappeared. In contrast, short-wavelength UV light did not reach the ground (Fig. 4(b)) when stratospheric temperature was below −75 °C, although the ozone hole had appeared (Fig. 4(a)). The largest amounts of short-wavelength UV light were found to reach the ground when the stratospheric temperature was in the range −65 °C to −56 °C, when the total amount of ozone in the sky began to increase (Fig. 4(b)).

Causal substances essential for ozone hole occurrence include fluorocarbons [15–17]. Ozone is destroyed by chlorine atoms, which are formed by the photo dissociation of fluorocarbons [15–17]. Extremely low temperatures lead to the formation of polar stratospheric clouds in the Antarctic stratosphere in winter. Chemical reactions readily take place on the surface of the polar stratospheric clouds, producing stable chlorine molecules from hydrogen chloride and chlorine nitrate. Sunlight irradiation in the spring transforms chlorine molecules to chlorine atoms, which extensively destroy ozone in the upper sky of the Antarctic. This is

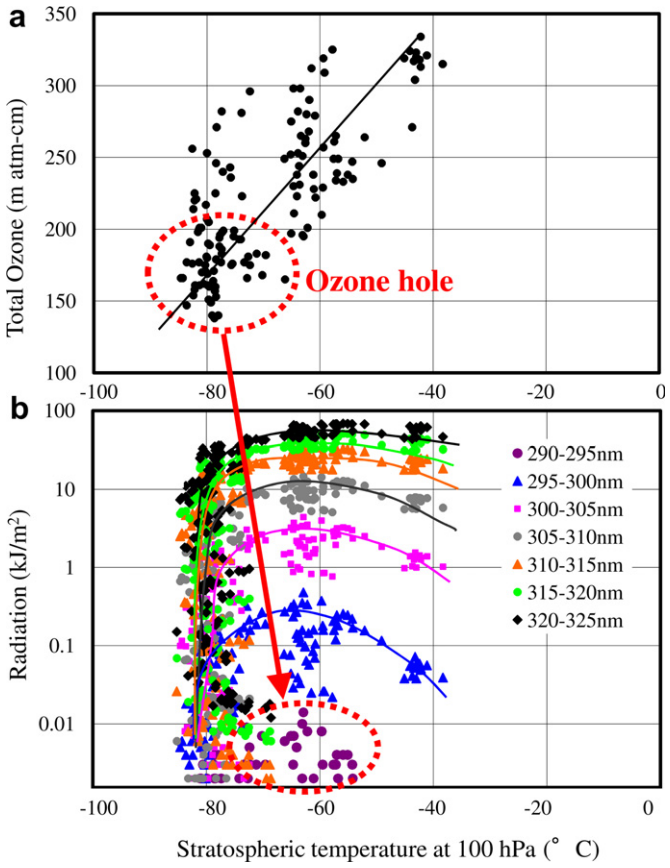

Fig. 4. Annual variations in daily representative data at Syowa Station from June to December 2007: (a) total amount of ozone versus stratospheric temperature, and (b) Ultraviolet radiation versus stratospheric temperature. The total amount of ozone refers to the sum of ozone present in all upper layers of the atmosphere above the observation point. The sum of ozone is converted to that at 0 °C and 1 atm, and expressed as thickness. Values expressed in "m atm-cm" were derived by multiplying values in "cm" by 1000.

why the widely recognized ozone hole occurs only in the spring [15–17].

It is generally believed that short-wavelength UV light readily reaches the ground surface from the sun because the total amount of ozone in the upper sky is small when the ozone hole occurs. However, little UV radiation reached the ground when the stratospheric temperature was below −75 °C, because the sun was low. In other words, it may be stated that a relatively small amount of short-wavelength UV (290–295 nm) radiation reached the ground because the amount of UV radiation from the sun was small when the stratospheric temperature was below −75 °C. In contrast, the sun was high and thus UV radiation levels were increased when the stratospheric temperature was in the range −75 °C to −55 °C. When the stratospheric temperature was −75 °C to −55 °C, solar radiation increased and thus increased levels of short-wavelength UV radiation (290–295 nm) reached the ground, although the total amount of ozone in the stratosphere above the Antarctic was larger than that measured when the stratospheric temperature was below −75 °C.

As described above, short-wavelength UV light (290–295 nm) was actually found to reach the ground when the stratospheric temperature was 10–15 °C higher than that measured when the ozone hole appeared. This was 1–2 months after the ozone hole began to appear. Considering the above results, 25 d from 11 September to 5 October 2007 and 50 d from 11 September to 30 October 2007 were chosen for exposure in the spring.

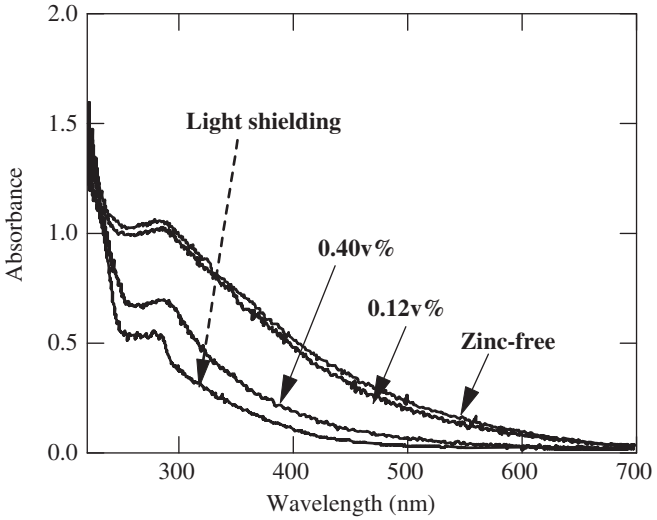

Fig. 5. Absorbances of collagen sheets covered with polypropylene films containing zinc oxide after spring sunlight exposure in the vertical direction (facing up).

3.2. Changes in the color of collagen sheets after exposure

To confirm the protective effect of a UV-cut material against short-wavelength UV light when the ozone hole appeared, collagen sheets covered with films containing zinc oxide particles were exposed outdoors. In the experiments, four types of polypropylene film containing various amounts of zinc oxide were used. To quantify color changes of the exposed collagen sheets, the spectrophotometer was used to measure absorbance spectra of reflected light. Fig. 5 presents the result of 50-d exposure in the vertical direction (facing up) in spring. The results show that when a collagen sheet covered with a zinc-free film was exposed, the absorbance was higher than that measured when a collagen sheet covered with a film containing zinc oxide was exposed, and discoloration was at its most intensive. However, a collagen sheet covered with a film containing zinc oxide at 0.12 v% showed relatively high absorbance. In contrast, when a collagen sheet covered with a film containing zinc oxide at 0.40 v% was exposed, the absorbance decreased substantially to a value quite close to that measured when a collagen sheet shielded with aluminum foil was exposed. In short, using a film containing zinc oxide at 0.40 v% to cover a collagen sheet could suppress chemical reactions such as oxidation in the collagen sheet, even when exposure lasts for 50 d in spring, when the ozone hole appears. This was inferred to be the reason behind the limited color change observed.

3.3. Protective effect of UV-cut film against short-wavelength UV light

Extracts were prepared from the collagen sheets exposed in the vertical direction (facing up) for 50 d and analyzed for amino acids.

Table 1
Amino acid amounts for extracts from collagen sheets covered with polypropylene films containing various zinc oxide content levels after sunlight exposure for 50 d of vertical exposure (facing up).

	Amounts of amino acids (μg/100 μl)	Zinc-free	0.04%	0.12%	0.40%	Light shielding
Autumn exposure	Total	139.7	123.5	61.8	38.2	11.1
	Hydroxyproline	17.13	15.25	7.60	4.37	1.15
Spring exposure	Total	384.3	287.1	325.6	91.5	10.4
	Hydroxyproline	44.55	34.27	38.56	11.10	1.01

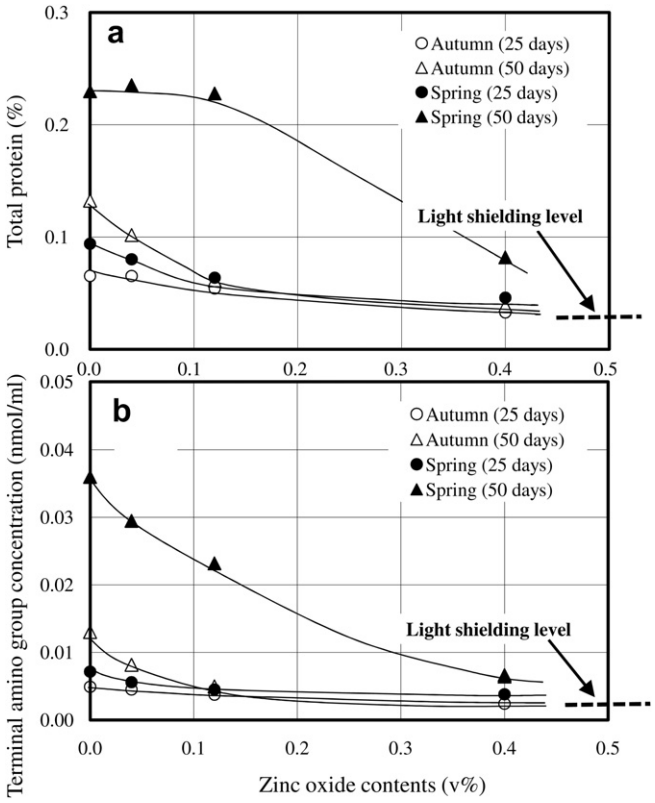

Fig. 6. Structural analysis of extracts from collagen sheets covered with polypropylene films containing zinc oxide after sunlight exposure in the vertical direction (facing up): (a) total protein, and (b) terminal amino group concentrations.

Table 1 lists the total amounts of detected amino acids and the amounts of hydroxyproline. The results indicate that both the total amount of amino acids and the amount of hydroxylproline were smaller in the collagen sheets covered with films containing larger amounts of zinc oxide. Furthermore, the value for exposure in spring, when the ozone hole began to appear, was found to be about 2.8 times as large as that for exposure in autumn. The data suggest that collagen molecular chains were more readily broken by high-energy UV light when the ozone hole began to appear. However, the total amount of amino acids in the collagen sheet covered by a film with a zinc oxide content of 0.40 v% decreased to nearly one-fourth that in a collagen sheet covered by a zinc-free film, even during the spring exposure.

Subsequently, extracts from the collagen sheets exposed in the vertical direction (facing up) for 25 d and 50 d were analyzed for total protein and terminal amino group concentrations (Fig. 6). The results show that total protein and terminal amino group concentrations decreased in all cases of exposure with increasing amounts of zinc oxide in the film. When the amount of zinc oxide added was 0.40 v%, total protein and terminal amino group concentrations decreased to their levels in the collagen sheets shielded with the aluminum foil, except for the 50-d spring exposure. In short, zinc oxide particles exhibited UV protection during exposure when the ozone hole appeared in the Antarctic. However, the collagen sheets degraded much more in the 50-d spring exposure than in the 25-d spring exposure and 25-d and 50-d autumn exposures. To obtain sufficient UV protection in the 50-d spring exposure, 0.40 v% or more of zinc oxide must be added to the film.

In addition, electrophoretic analysis was performed with extracts from the collagen exposed in the vertical direction (facing up) for 50 d. The left-hand gel in Fig. 7 shows the results of the autumn exposure, and the right-hand gel indicates the results of the spring exposure. The results show that exposed sheets had more collagen degradation products than sheets (①, ②) shielded from UV. Spring exposure yielded more degradation products than

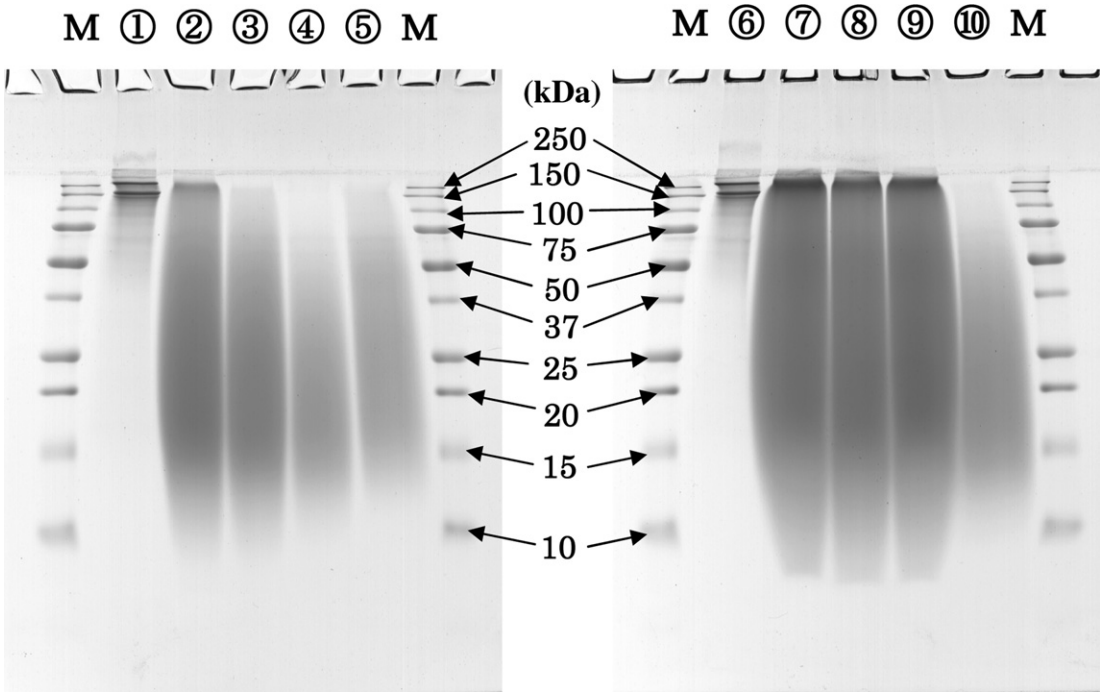

Fig. 7. SDS-PAGE of extracts from collagen sheets covered with polypropylene films containing various levels of zinc oxide after sunlight exposure for 50 d in a vertical direction (facing up): left: autumn exposure, and right: spring exposure. ① light shielding (autumn), ⑥ light shielding (spring), ②⑦ zinc-free, ③⑧ 0.04 v%, ④⑨ 0.12 v%, and ⑤⑩ 0.40 v%; M: molecular marker.

autumn exposure. Among collagen sheets subjected to spring exposure, the sheets covered with a film containing zinc oxide at 0.40 v% had smaller amounts of degradation products than the sheets covered with other films. In short, the film with a zinc oxide content of 0.40 v% had a high UV protection effect. As explained above, the results of electrophoresis also showed that zinc oxide particles in the film effectively protect collagen molecule chains from short-wavelength UV light in the Antarctic when the ozone hole appears.

3.4. Differences resulting from exposure direction

The sun is very low, in the range 8–25°, even at noon in spring and autumn in the Antarctic. Moreover, a person standing erect receives low-angled solar radiation on their face. Therefore, a test was performed for exposure not only in the vertical direction (facing up) but also in the horizontal direction (facing true north). Fig. 8 shows total protein and terminal amino group concentrations of collagen sheets exposed in the horizontal direction (facing true north). These results suggest that total protein and terminal amino group concentrations were close to those for exposure in the vertical direction (facing up) shown in Fig. 6. In other words, both total protein and terminal amino group concentrations decreased significantly with increasing zinc oxide content in all cases of exposure. At a zinc oxide content of 0.40 v%, total protein and terminal amino group concentrations decreased to those of exposed collagen sheets that were shielded with aluminum foil, except for 50-d exposure in the spring.

A previous paper [10] on summer exposure reported that terminal amino group concentrations were about three times higher in the case of vertical exposure (facing up) than in the case of horizontal exposure (facing true north). However, this study indicated few differences between the two exposure directions in both spring and autumn. In other words, the effect of vertical exposure (facing up) relative to that of horizontal exposure (facing true north) was found to be smaller in spring and autumn than in summer. It is believed that this is a result of the lower height of the sun in spring and autumn than in summer. Consequently, the relative effect of exposure in the vertical direction (facing up) diminishes.

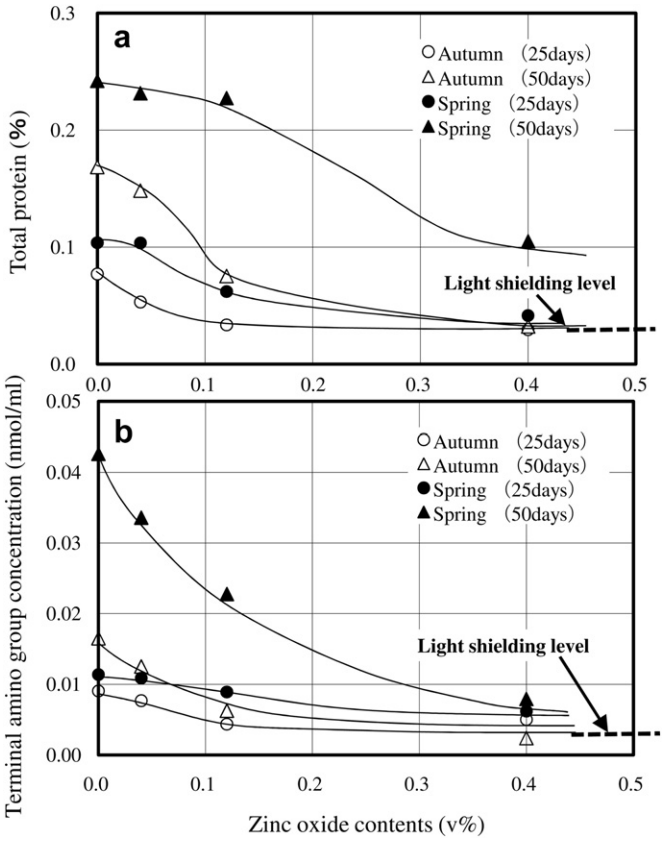

Fig. 8. Structural analysis of extracts from collagen sheets covered with polypropylene films containing zinc oxide after sunlight exposure in the horizontal direction (true north): (a) total protein, and (b) terminal amino group concentrations.

4. Conclusion

Polypropylene films with various amounts of zinc oxide were prepared to cover simulated human skin in the form of collagen sheets. The covered collagen sheets were exposed in the Antarctic when the ozone hole appeared there.

(1) Zinc oxide added to the film at 0.40 v% suppressed the deterioration of exposed collagen sheets substantially and kept color changes relatively small in collagen sheets exposed for 50 d in spring, when the ozone hole appeared.
(2) Exposed collagen sheets were dissolved to measure total amino acid amounts; the amount was 2.8 times higher in the case of exposure in the spring than in the case of exposure in the autumn. However, the total amount of amino acid decreased in the collagen sheet covered by a film with a zinc oxide content of 0.40 v% to nearly one-fourth that in a collagen sheet covered by a zinc-free film, even during spring exposure.
(3) Analysis shows that total protein and terminal amino group concentrations decreased substantially in extracts from collagen sheets with the addition of increasing levels of zinc oxide to the films. In other words, the addition of zinc oxide was confirmed to suppress collagen deterioration by UV light very effectively, even if exposure lasted 50 d in spring, when the ozone hole appeared.
(4) Differences resulting from exposure direction were also studied. The results suggest that collagen sheet deterioration was approximately three times greater in the case of vertical exposure (facing up) than in the case of horizontal exposure (facing true north) in summer, whereas few differences were found between the two exposure directions in spring and autumn. In other words, compared with collagen sheets exposed in the summer, relative deterioration of collagen sheets by horizontal exposure (facing true north) to collagen sheet deterioration by vertical exposure (facing up) was found to be greater in the spring and the autumn. The addition of zinc oxide to the films was also very effective in the case of exposure in the horizontal direction (facing true north).

References

[1] Chubachi S. Preliminary result of ozone observation at syowa station from February 1982 to January. Mem Natl Inst Polar Res; 1983:13–9 [Spec Issue: 34].
[2] Farman JC, Gardiner BG, Shanklin JD. Large losses of total ozone in Antarctica reveals seasonal ClOx/NOx interaction. Nat (London U K.) 1985;315:207–10.
[3] Stolarski RS, Krueger AJ, Schoeberl MR, McPeters RD, Newman PA, Alpert JC. Nimbus 7 satellite measurements of the spring time Antarctic ozone decrease. Nat (London U K.) 1986;322:808–11.
[4] Miyagawa K. Vertical ozone profile by umkehr measurements at syowa station. Int J Remote Sens 2009;30(15/16):4043–53.
[5] Newman PA, Kawa SR, Nash ER. On the size of the Antarctic ozone hole. Geophys Res Lett 2004;31(21):L21104.1–4.
[6] Mommaas AM. Effects of ultraviolet radiation on human skin. Sunburn-photoaging-immunosuppression-cancer. Seifen Oele Fette Wachse 1998;124(13): 886–8. 882, 884.
[7] Pitari G, Visconti G, Verdecchia M. Global ozone depletion and the Antarctic ozone hole. J Geophys Res 1992;97(D8):8075–82.
[8] Zhao Y, Kondo Y. Wintertime stratospheric ozone changes over Japan since 1991. Geophys Res Lett 1996;23(15):1969–72.

[9] Takahashi T, Tanaka K, Hattori S, Irie S, Kudoh S, Imura S, et al. Evaluation of UV protection effect for UV-cut materials using the collagen artificial skin. Sen'i Gakkaishi 2009;65(12):344–50.
[10] Takahashi T, Yamamoto T, Kasai W, Kondo T, Tanaka K, Hattori S, et al. Protection effect for collagen artificial skin of UV-cut materials in Antarctica. Sen'i Gakkaishi 2009;65(12):351–8.
[11] Submitted to Journal of Photochemical & Photobiological Sciences.
[12] Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nat (London U K.) 1970;227:680–5.
[13] Ebihara T, Iijima K, Sato K, Someki I, Kuwahara K, Hattori S, et al. Change in the extractability of bovine skin collagen through the course of aging and biochemical properties of the collagen extracted. Connect Tissue 1999;31:17–23.
[14] Japan Meteorological Business Support Center. Antarctic meteorological record cd-rom; 2009.
[15] Solomon S, Garcia RR, Rowland FS, Wuebbles DJ. On the depletion of Antarctic ozone. Nat (London U K.) 1986;321:755–8.
[16] Tung Ka-Kit, Ko MW, Rodriguez JM, Sze ND. Are Antarctic ozone variations a manifestation of dynamics or chemistry? Nat (London U K.) 1986;322:811–4.
[17] Molina LT, Molina MJ. Production of the Cl_2O_2 from the self-reaction of the ClO radical. J Phys Chem 1987;91:433–6.