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Utilization of Salmon Milt DNA Against UV Damage

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Abstract We examined the effect of ultraviolet (UV) irradiation on the UV spectra and radical scavenging activity of DNA strands and found that the absorption spectra of salmon milt DNA was extended up to about 350 nm after ultraviolet C (UVC, 100–280 nm) irradiation with 300 kJ/m^2. The UV B (UVB, 280–315 nm) protection ability of UVC-irradiated salmon milt DNA for a single-stranded target DNA (19-mer) was further studied. The percentage of damaged target DNA after 50 kJ/m^2 of UVB irradiation in the presence of UVC-irradiated salmon milt DNA, UVC-unirradiated salmon milt DNA, and 2-phenylbenzimidazole sulfonic acid was estimated to be 24.6%, 27.0%, and 18.9%, respectively. Moreover, the ultraviolet A (UVA, 315–400 nm)/UVB ratio and critical wavelength of natural (UVC-unirradiated) salmon milt DNA were estimated to be 0.13 and 313 nm, respectively, whereas those of the UVC-irradiated salmon milt DNA were 0.34 and 375 nm, respectively. Interestingly, the value of 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity in UVC-irradiated salmon milt DNA was about five times higher than that of UVC-unirradiated salmon milt DNA. These results indicate that the UVC-irradiated salmon milt DNA could be useful as a protector against a wide range of UV light from UVC~UVA.

Keywords Ultraviolet C-irradiated salmon milt DNA · Ultraviolet C-unirradiated salmon milt DNA · Ultraviolet absorption · Ultraviolet B protection ability · 1,1-diphenyl-2-picrylhydrazyl radical scavenging activity

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Introduction

DNA, which is a natural polymer and one of the most important materials in living organisms, has unique chemical structures and various biological functions [1]. Recently, since several researchers have reported the physiological functionality of DNA from salmon milt by oral administration [2–4], salmon milt DNA has created interest in utilization as a health food. However, large amounts of this DNA source are still being discarded. For recycling this industrial waste, we have studied the utilization of the DNA as a functional biomaterial. Since DNA is odorless, colorless, has low permeability into skin owing to its anionic water-soluble properties, and has ultraviolet (UV) absorption ability [1, 5], we focused on the utilization of DNA as a cosmetic against UV damage. To our knowledge, although DNA is already being mixed with some cosmetics, there have been few efforts to demonstrate the functionality of DNA as a UV protectant.

Solar radiation causes sunburns in human skin and cellular DNA damage by direct excitation of DNA (formation of DNA photoproducts) and indirect mechanisms that involve the excitation of other cellular chromophores and oxidative DNA modification [6–11]. Thus, it is important to demonstrate the UV protection ability and the radical scavenging activity of salmon milt DNA in order to use it as a functional cosmetic.

On the other hand, the maximum UV absorption (around 260 nm) of natural DNA is located in the UVC area, which is mostly blocked by the atmosphere [12], making crude DNA difficult to use as an efficient UV protector. In addition, there are no data in the literature for the radical scavenging activity of naturally occurring DNA. The UV absorption ability and radical scavenging activity of DNA is dependent on the electron states of the DNA bases. Although it has been reported that several metal ions (Ag^{+}, Cu^{2+}, Hg^{+}, and Au^{2+}) are able to change the absorption spectrum by binding to DNA bases [13–16], the use of these metal ions in a UV protector is not desirable because of their safety and cost. Thus, to change the electronic states of the DNA bases, we studied here the effect of irradiation with UVC on the UV absorption properties and radical scavenging activity of salmon milt DNA. We found that the UV absorption of salmon milt DNA was shifted to a longer wavelength by UVC irradiation. UVB protection ability, UVA absorption ability, and DPPH radical scavenging activity tests showed that the UVC-irradiated salmon milt DNA is more effective than natural salmon milt DNA.

Materials and Methods

Materials

Salmon milt DNA (Na salt, double-stranded DNA, molecular weight=$5\times10^{4}\sim2\times10^{5}$) was purchased from Croda Japan (Tokyo, Japan). HPLC-grade target DNA (5′-TGCACG CATTGCAGGTAGC-3′), Oligo1 (5′-TGCACGCAATGCAGGTAGC-3′), and Oligo2 (5′-GCTACCTGCATTGCGTGCA-3′) were purchased from Hokkaido System Science (Sapporo, Japan). The target DNA was labeled with 6-carboxyfluorescein (FAM) at the 5′ ends and used to investigate the effect of natural (UVC-unirradiated) and UVC-irradiated salmon milt DNA on thymine dimer formation induced by UVB irradiation. Reagent grade 2-phenylbenzimidazole sulfonic acid and DPPH 6-hydroxy-2, 5, 7, 8-tetramethyl-2-carboxylicacid (Trolox, a water soluble homologue of vitamin E) were purchased from Wako Pure Chemicals (Osaka, Japan) and used without further purification.

UVC Irradiation

Salmon milt DNA was dissolved in phosphate-buffered saline (PBS buffer: 137 mM NaCl, 8.1 mM Na_2HPO_4, 2.68 mM KCl, 1.47 mM KH_2PO_4, pH 7.4) and irradiated in quartz cuvettes of 1-cm light-path containing 3 mL solution at room temperature. UVC irradiation (254 nm) was applied with an Ultraviolet Crosslinker CX-2000 (UVP, CA, USA) with a 40-W GL8 bactericidal lamp (Sankyo Denki, Tokyo, Japan). UV absorption spectra were measured on a BECMAN DU640 spectrophotometer (Beckman Coulter, CA, USA).

CD Measurement

CD spectra were recorded using a Jasco J-820 spectrometer equipped with a Peltier temperature control system (Jasco, Tokyo, Japan). The cuvette-holding chamber was flushed with a constant steam of dry N_2 gas to avoid water condensation on the cuvette exterior. CD spectra data were collected from 220 to 400 nm with a 4-s response time and a 1-nm bandwidth using a 0.1-cm quartz cuvette. CD measurements for 50 μg/mL salmon milt DNA were carried out in PBS buffer (pH 7.4) with or without UVC irradiation (300 kJ/m^2). Each spectrum shown in this study is the average of five individual scans.

Measurement of Protection Ability of Salmon Milt DNA Against Thymine Dimer Formation of Target DNA Induced by UVB

The protection ability of UVC-unirradiated salmon milt DNA and UVC-irradiated salmon milt DNA against thymine dimer formation for target DNA was assayed in PBS in the absence or presence of the UVC-unirradiated salmon milt DNA, UVC-irradiated salmon milt DNA, or 2-phenylbenzimidazole sulfonic acid; 50 kJ/m^2 of UVB irradiation was conducted at room temperature. Mixtures of damaged and undamaged target DNAs after UVB irradiation were separated by 20% native polyacrylamide gel electrophoresis (PAGE). The native PAGE was run at 200 V and visualized using a Fujifilm FLA-5100 phosphorimager (Fujifilm Co., Tokyo, Japan). Bands were quantified by measuring fluorescence intensity (LAU/mm^2) using Multi Gauge ver. 2.2 software (Fujifilm Co.). The UVA/UVB ratio and the critical wavelength of salmon milt DNA with or without UVC irradiation were measured with a UV-1000S ultraviolet transmittance analyzer (Labsphere, NH, USA).

DPPH Radical Scavenging Assay

The effect of DNAs on DPPH radicals was determined using the method of Yamaguchi et al. [17]. A solution of 0.2 mM DPPH in ethanol was prepared, and 200 μL of this solution was mixed with 1 mg/200 μL of DNA in 100 mM MES buffer (pH 7.0) for each type of DNA. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 20 min. The absorbance of the mixture was measured spectrophotometrically at 517 nm. Trolox was used as a reference. The ability to scavenge DPPH radicals was presented as Trolox equivalents. One milligram salmon milt DNA, UVC-irradiated salmon milt DNA, Oligo1, Oligo2, and 19-mer double-stranded DNA (dsDNA) were used in this study. The 1.7 mM (1 mg) dsDNA was prepared by annealing (incubation at 90 °C for 5 min, followed by cooling at 1 °C/min to the reaction temperature) of 850 μM Oligo1 with 850 μM Oligo2.

Results and Discussion

Effect of UVC Irradiation on the Spectroscopic Properties of Salmon Milt DNA

Since light energy causes a chemical reaction (damage) in DNA bases [6, 10], we first studied the UV absorption and the CD spectra change in salmon milt DNA induced by UVC irradiation. The UV absorbance of 50 μg/mL salmon milt DNA in PBS before and after UVC irradiation (300 kJ/m^2) at room temperature is shown in Fig. 1a. Although the absorbance at 260 nm of salmon milt DNA decreased with UVC irradiation, the absorption area was extended to a longer wavelength. With an increase in the salmon milt DNA concentration from 50 to 1000 μg/mL, further extension of the absorption range induced by UVC irradiation was not observed (data not shown). This observation is explained by the fact that the same numbers of photons are absorbed in all cases; therefore, the same amount of damage is generated. However, because the amount of unmodified DNA is much larger for a concentrated solution, the UV absorption is not significantly modified. In addition, the absorbance of 50 μg/mL of salmon milt DNA around 300–350 nm increased with UVC irradiation up to 300 kJ/m^2 (data not shown). From these results, we set the conditions to extend the absorption range of salmon milt DNA at 50 μg/mL DNA concentration and 300 kJ/m^2 UVC irradiation.

Next, to investigate the mechanism of the extension of the absorption range of salmon milt DNA, we examined the DNA structures before and after UVC irradiation. CD spectra of the salmon milt DNA in PBS (pH 7.4) before and after UVC irradiation (300 kJ/m^2) at 25 °C are shown in Fig. 1b. The CD spectrum of UVC-unirradiated salmon milt DNA showed a typical B-form signal with positive peaks around 280 and 220 nm and a negative peak around 245 nm. After UVC irradiation, the intensity of these peaks decreased and a weak negative peak area rose around 300–345 nm. These changes of CD spectra after UVC irradiation are similar with those observed previously for calf thymus DNA and indicate that the double helix structure of salmon milt DNA was changed by the formation of pyrimidine dimers [18]. In addition to pyrimidine dimers, UV light is absorbed by nucleobases in DNA and induces typical photochemical reactions. It has been reported that the major DNA changes by UV radiation are the cis-syn cyclobutane pyrimidine dimer (CPD) and the pyrimidine (6-4) pyrimidone photoproduct ((6-4) photoproduct) formed at

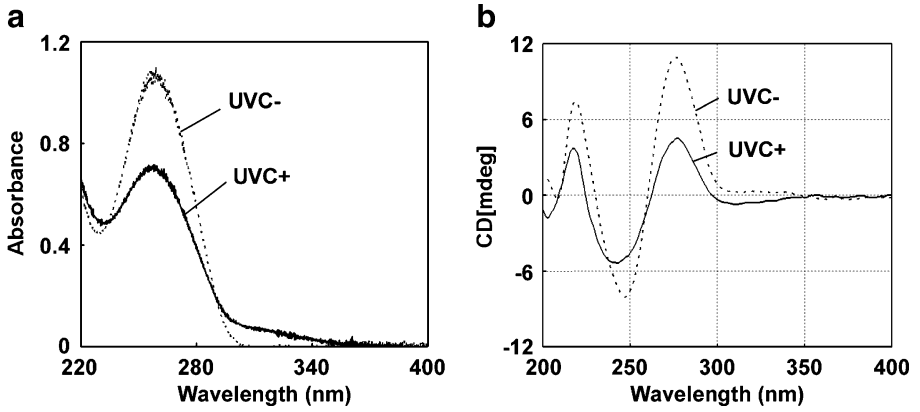

Fig. 1 **a** UV and **b** CD spectra of salmon milt DNA in PBS buffer (pH 7.4) before (*broken line*, UVC−) and after (*solid line*, UVC+) UVC irradiation at room temperature

adjacent pyrimidine bases [19–22]. However, it has been known for decades that under high UV doses such as used in our study, photoproducts are formed at adjacent adenine bases [23–26]. Although CPDs are the most frequently observed photoproducts induced by UV irradiation, Setlow reported that the absorbance of thymine dimers shifts the spectrum to a shorter wavelength [21], which is inconsistent with the UV results obtained here (Fig. 1a). On the other hand, it has been shown that the (6-4) photoproduct and the adenine photoproduct exhibit a strong absorption around 320 and 310 nm, respectively [19, 25]. Thus, the extension of the absorption area of salmon milt DNA observed in this study after UVC irradiation can be attributed to (6-4) photoproducts and adenine photoproducts. Since the (6-4) photoproduct is produced with a quantum yield higher than the adenine photoproduct [18, 24], it is reasonable to conclude that the (6-4) photoproduct is the major factor of the extension of the absorption area.

Protection Ability of UVC-Irradiated Salmon Milt DNA Against UVB Damage of Target DNA

UVB irradiation generates predominantly thymine dimers, which lead to the cytotoxic, mutagenic, and carcinogenic effects of UVB radiation [27–30]. Thus, the protection ability of UVC-irradiated salmon milt DNA against thymine dimer formation induced by UVB irradiation should be studied. We tested this effect on a target DNA irradiated with UVB. Figure 2 shows native PAGE results for the target DNA after UVB irradiation in the presence of 0-, 80-, 400-, or 800-μg UVC-irradiated salmon milt DNA. Since a kink in a DNA strand is caused by cross-linked bases, DNA strands with thymine dimers migrate more slowly than those without [31]. The intensity of the upper DNA band, which corresponds to damaged target, decreased with increasing amounts of UVC-irradiated salmon milt DNA. Damaged target DNA bands were hardly observed in the presence of 400- and 800-μg UVC-irradiated salmon milt DNA. These results demonstrate that UVC-irradiated salmon milt DNA can protect the target DNA from damage induced by UVB irradiation.

The UVB protection ability of UVC-irradiated salmon milt DNA was further compared with that of UVC-unirradiated salmon milt DNA and 2-phenylbenzimidazole sulfonic acid, very commonly used as a water soluble sunscreen [32]. The ratio of the damaged DNA to the total target DNA in the absence or presence of 50 μg of each UVB protector is shown in Fig. 3. The percentage of damaged DNA was estimated by the intensities of the damaged and total target DNA bands (Fig. 2). In the absence of UVB protectors, the percentage of the damaged target DNA after 50 kJ/m^2 UVB irradiation was estimated to be 34.6%. On the other hand, the percentage of the damaged target DNA after irradiation in the presence of

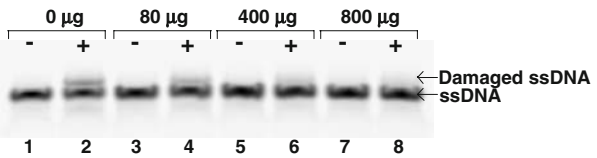

Fig. 2 Effect of the photoproduct of salmon milt DNA on the thymine dimer production of 19-mer ssDNA. UVB irradiation (50 kJ/m^2) was conducted in PBS buffer (pH 7.4) containing 10 μM (6.4 μg) 19-mer ssDNA at room temperature. *Lanes 1*, *3*, *5*, and *7* (*minus sign* before UVB irradiation) show nondamaged 19-mer ssDNA bands in the presence of 0, 80, 400, and 800 μg photoproduct of salmon milt DNA, respectively. *Lanes 2*, *4*, *6*, and *8* (*plus sign* after UVB irradiation) show damaged 19-mer ssDNA bands in the presence of 0, 80, 400, and 800 μg photoproduct of salmon milt DNA, respectively

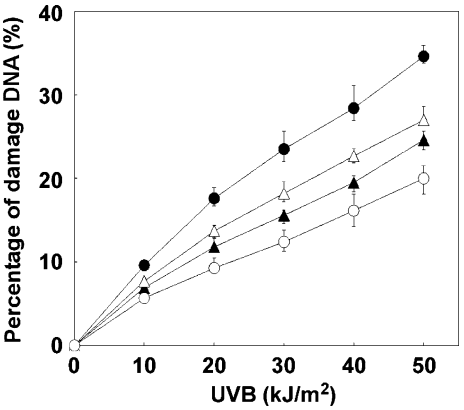

Fig. 3 The percentage of damage DNA in the absence (*closed circles*) or presence of 50 μg UVC-unirradiated salmon milt DNA (*open triangles*), 50 μg UVC-irradiated salmon milt DNA (*closed triangles*), and 50 μg 2-phenylbenzimidazole sulfonic acid (*open circles*). UVB irradiation was conducted in PBS buffer (pH 7.4) containing 10 μM (6.4 μg) 19-mer ssDNA at room temperature. The percentage of damaged DNAs was estimated using the following equation: the percentage of damage DNA=100×[fluorescence intensity (LAU/mm^2) of the damaged 19-mer ssDNA band]/[fluorescence intensity (LAU/mm^2) of the total 19-mer ssDNA band]

UVC-irradiated salmon milt DNA, UVC-unirradiated salmon milt DNA, or 2-phenylbenzimidazole sulfonic acid was estimated to be 24.6%, 27.0%, and 18.9%, respectively. The UVB protection ability of UVC-irradiated salmon milt DNA is higher than that of UVC-unirradiated salmon milt DNA. This result should be due to the extension of the absorption spectrum of salmon milt DNA to the UVB area by UVC irradiation. The extinction coefficients of UVC-irradiated salmon milt DNA, UVC-unirradiated salmon milt DNA, and target DNA in the UVC (260~270 nm) and UVB (280~315 nm) regions are listed in Table 1. The extinction coefficients of UVC-irradiated salmon milt DNA at 310 and 315 nm were calculated to be 1.0 and 0.9 $cm^{-1}g^{-1}L$, respectively. In contrast, the extinction coefficients of UVC-unirradiated salmon milt DNA and target DNA were estimated to be zero since they did not have absorbance at those wavelengths. These results

Table 1 The extinction coefficient of UVC-irradiated salmon milt DNA and target DNA in each wavelength at room temperature.

Wavelength (nm)	Extinction coefficient ($cm^{-1}g^{-1}L$)		
	UVC-irradiated salmon milt DNA	UVC-unirradiated salmon milt DNA	Target DNA
260	14.8	24.2	26.5
270	13.5	22.8	21.8
280	10.4	14.3	13.9
290	4.2	6.5	6.1
300	1.4	0.9	0.7
310	1.0	0	0
315	0.9	0	0

Since the molecular weight of the salmon milt DNA is not clear, the unit of the extinction coefficient was shown by weight (cm^{-1} g^{-1} L)

indicate that the UVB protection ability of UVC-irradiated salmon milt DNA shown in Fig. 3 is due to its absorbance from 280 to 300 nm. Moreover, UVC-irradiated salmon milt DNA can absorb at all UVB regions from 280 to 315 nm. Although 2-phenylbenzimidazole sulfonic acid showed the most potent UVB protection ability, our result showed that UVC-irradiated salmon milt DNA also efficiently inhibits thymine dimer formation induced by UVB irradiation in target DNA.

UVA Protection Ability of UVC-Irradiated Salmon Milt DNA

Since the mechanism of DNA damage with UVA irradiation is different from that of UVB irradiation [9], we further investigated the UVA protection ability of the photoproduct of salmon milt DNA. The UVA/UVB ratio and critical wavelength are important parameters to evaluate the UVA protection ability for human skin of a UV protector; the UVA/UVB ratio is defined as the ratio of the means of absorbances from UVA and UVB, and the critical wavelength is the point where 90% of the area under the curve lies, starting at the UVB end (290 nm) [33]. Table 2 lists the calculated UVA/UVB ratios and critical wavelengths of salmon milt DNA with or without UVC irradiation. The values of the UVA/UVB ratio and critical wavelength of salmon milt DNA without UVC irradiation were 0.13 and 313 nm, respectively, whereas those of the photoproduct of salmon milt DNA were 0.34 and 375 nm, respectively. The UVA/UVB ratio of the salmon milt DNA increased with UVC irradiation and the critical wavelength was shifted to a longer wavelength. These results show that the UVA protection ability for human skin of the photoproduct of salmon milt DNA is stronger than that of natural salmon milt DNA and that the photoproduct of salmon milt DNA can reduce damage not only by UVB irradiation but also by UVA irradiation.

DPPH Radical Scavenging Activity of UVC-Irradiated Salmon Milt DNA

UVA irradiation generates reactive oxygen species such as singlet oxygen, hydroxyl radicals, and superoxide, which cause DNA damage through indirect mechanisms such as the formation of 8-hydroxyguanine [9]. Thus, radical scavenging activity is one of the important functional factors in the utilization of salmon milt DNA against UV damage. We, thus, tested the radical scavenging activity of UVC-irradiated salmon milt DNA. The DPPH radical scavenging activity of UVC-irradiated salmon milt DNA, UVC-unirradiated salmon milt DNA, Oligo1, Oligo2, and dsDNA with Oligo1 and Oligo2 is shown in Fig. 4. Surprisingly, the DPPH radical scavenging activity of UVC-irradiated salmon milt DNA (126.1 μM/mg Trolox equivalent) was significantly higher than that of UVC-unirradiated salmon milt DNA (25.8 μM/mg Trolox equivalent). This result indicates that UV irradiation of DNA causes an enhancement of radical scavenging activity.

Table 2 UVC irradiation effects on the UVA/UVB ratio and critical wavelength of DNA.

	UVA/UVB ratio	Critical wavelength (nm)
UVC^- DNA[a]	0.13	313
UVC^+ DNA[a]	0.34	375

The value of the UVA/UVB ratio and the critical wavelength is the average of ten individual scans

[a] 20 mg/mL of the photoproduct of salmon milt DNA with UVC irradiation (300 kJ/m^2)

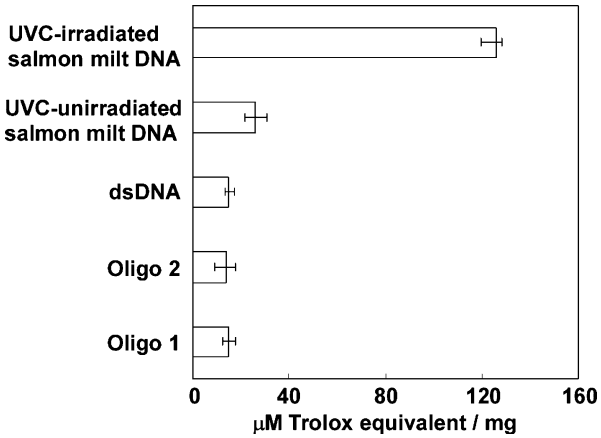

Fig. 4 DPPH radical scavenging activity in 1 mg UVC-irradiated salmon milt DNA, UVC-unirradiated salmon milt DNA, Oligo1 (1.7 mM), Oligo2 (1.7 mM), and dsDNA (1.7 mM) with Oligo1 (850 μM) and Oligo2 (850 μM). The ability to scavenge DPPH radicals was presented as μM/mg Trolox equivalent

UV irradiation of DNA not only causes the formation of photoproducts but also causes strand breaks [34]. In fact, we observed the production of low molecular weight DNA fragments by UVC irradiation for salmon milt DNA with agarose gel electrophoresis (data not shown). Thus, we examined whether the strand length and the global structure of DNA affect DPPH radical scavenging activity using Oligo1, Oligo2, and dsDNA. DPPH radical scavenging activity of 1 mg (1.7 mM) Oligo1 and 1 mg (1.7 mM) Oligo2 (19-mer single-stranded DNAs) were 14.8 μM/mg Trolox equivalent and 13.7 μM/mg Trolox equivalent, respectively. In addition, the DPPH radical scavenging activity of 1 mg (1.7 mM) dsDNA (19-mer double-stranded DNA) showed a value similar to that of Oligo1, Oligo2, and UVC-unirradiated salmon milt DNA. These results indicate that the strand length and the helical structure of DNA are not critical factors in DPPH radical scavenging activity. On the other hand, since the effect of antioxidants on DPPH is thought to be due to their hydrogen- or electron-donating ability [35], the enhancement of DPPH radical scavenging activity in UVC-irradiated salmon milt DNA may be due to the enhancement of the proton or electron donating ability of the photoproducts formed in salmon milt DNA by UV irradiation.

In conclusion, we found that UVC-irradiated salmon milt DNA can inhibit thymine dimer formation in target DNA induced by UVB irradiation and can enhance DPPH radical scavenging activity. Although further studies should be conducted to confirm the safety of damaged DNAs, it is reasonable to conclude that UVC-irradiated salmon milt DNA is useful as a UVB and UVA protector and antioxidant. Since it has advantages such as odorlessness, colorlessness, low permeability in skin owing to the anionic water-soluble character of long DNA strands, and is an inexpensive material, use of the photoproduct of salmon milt DNA as a wide wavelength range UV protector could be promising not only for human skin but also for many other applications such as a UV protector for crops and pet animals.

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