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ESR Study of Disulfide Neutral Radical of α-Keratin Present in Dried White Human Hair Exposed to Near-UV Radiation

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ESR spectrum attributed to a α-keratin-derived disulfide neutral radical, which is formulated to be RSSHR$^{\bullet}$ ($g_1 = 2.060$, $g_2 = 2.026$, $g_3 = 2.000$, and $A_2 = 0.8$ mT), is observed for completely dried white human hair exposed to near-UV radiation. It is also observed that the residual water present in the matrix of human hair markedly accelerates the decomposition of disulfide radicals.

It is well known that both the structure and the biological functions of proteins change when they are exposed to near-UV radiation.[1] It is also known that the photoinduced splitting of the disulfide group is one of the major causes for the photodegradation of proteins.[2] Further, the photoexcited state of tryptophan or tyrosine has been regarded as a possible electron source for the reductive cleavage of the disulfide linkage.[3] The anion radical of the disulfide group or its protonated form has been speculated to be a transient intermediate species. Thus far, ESR measurements have been conducted for cystein-rich proteins, such as α-keratin of human hair and lamb wools; however, only a few reports on the observation of a well-resolved ESR spectrum have been published.[4] In the present study, ESR measurements are carefully performed on human hair exposed to near-UV radiation in order to detect α-keratin-derived disulfide radicals.

Commercially available untreated white human hair (Beaulax Co., Ltd.) was used, so as to neglect ESR signal arising from melanin pigment.[5] The hair was washed with 5.0% sodium dodecyl sulfate, rinsed in pure water, and stored over P_2O_5 for five days.[6] The water content in the hair (hereafter referred to as Wd) was evaluated to be 4.5% (in weight), which is the minimum water content of human hair,[6] by using a differential thermal analyzer (Rigaku, TG8101D). A lock of 100 Wd hair (33 mm, 19.0–21.0 mg) was bundled up with a copper film ($3.0 \times 8.0 \times 0.1$ mm^3), and this bundle was sealed in a quartz ESR tube under dry helium. A white human hair sample containing 12% water (hereafter referred to as Wa), which is the normal water content in human hair, was also prepared by an analogous procedure to that described above. The bundled sample was frozen at 77 K in a quartz dewar (JEOL) and was exposed to near UV-radiation for 30 min by using a Xe-lamp (UVF-203S, Sanei Electric, 150 W, wavelength from 255 to 390 nm), equipped with a band-pass filter (RU-340, Sanei Electric). ESR spectra were recorded by using an X-band ESR spectrometer (JEOL, TE-300) at 77 K. 2,2,6,6-tetramethyl-4-hydroxylpiperidine-1-oxyl (Sigma Aldrich) was used as the primary standard of radical concentration. ESR parameters were estimated as a mean value of three independent ESR measurements.

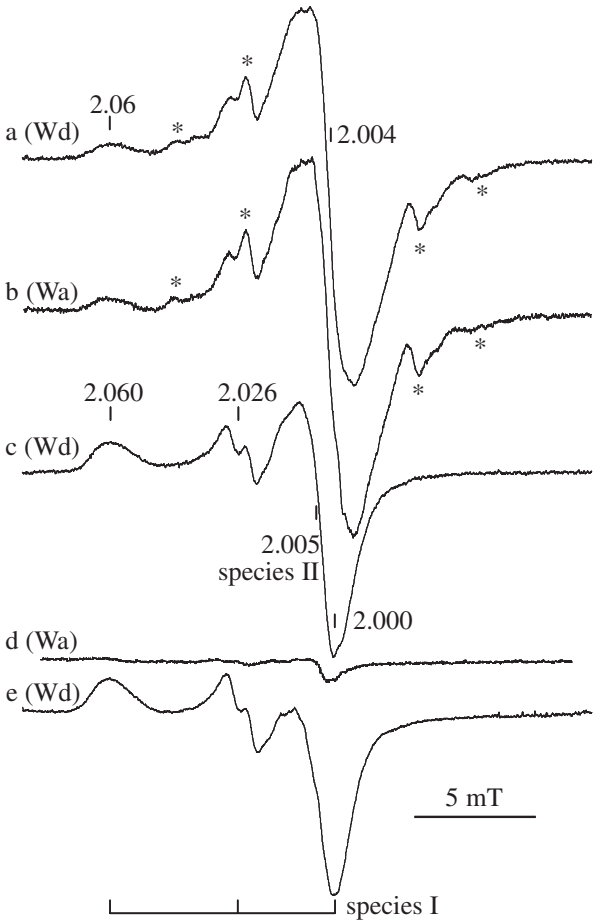

Figure 1. ESR spectra observed at 77 K (a) for Wd after near-UV radiation, (b) for Wa after near-UV radiation, (c) for Wd after annealing at 296 K for 30 min, (d) for Wa after the annealing treatment, and (e) for Wd by changing the microwave power to 5.0 mW. The asterisks indicate proton hyperfine splitting derived from fatty acids.

ESR spectra recorded for Wd and for Wa at 77 K showed a weak isotropic ESR signal due to the organic radical at $g = 2.005$ (data not shown). ESR spectrum was again recorded after exposing Wd to near-UV irradiation (0.78 W cm^{-2}) at 77 K for 30 min.[7] As shown in Figure 1a, the observed ESR spectrum showed the presence of a strong ESR signal ($g = 2.004$), with weak signals arising from proton hyperfine splitting. In addition, a part of an anisotropic ESR signal was also detected at a low magnetic field at $g = 2.06$. On the basis of the g-value and the ESR line shape, it was confirmed that the major paramagnetic

species were ascribed to be the carbon-centered radicals of fatty acids located on the surface of human hair.[8] Similar ESR measurements were also performed for Wa after similar near-UV radiation. As shown in Figure 1b, the observed ESR spectrum was almost identical to that recorded for Wd (Figure 1a), indicating that the excess water did not significantly disturb the formation of the carbon-centered radicals and of the anisotropic species.

Next, the near-UV-irradiated Wd was annealed by being thawed at 296 K, stored for 30 min, and gain supplied for ESR measurement. ESR signals attributed to the anisotropic species were clearly recorded, with the disappearance of the carbon-centered radical, as depicted in Figure 1c. The signal intensity of the radicals decreased only slightly, after the sample was allowed to stand at 296 K for 3 h. In addition, the total spin concentration was evaluated to be 6.0×10^{17} (spin g^{-1}), which corresponded to ca. 0.14% of the half-cystine content of human hair (1435 μmol g^{-1}).[8] This result provides experimental evidence that the quantum yield for the formation of the radicals is quite low, under the present conditions.

Further ESR measurements were performed for the frozen Wa, with the objective of examining the effect of free water on the ESR spectrum. Unlike that observed in the case of Wd, the anisotropic ESR signals almost disappeared, during the annealing treatment at 296 K for 30 min, as shown in Figure 1d. This result indicates that the residual moisture in the hair markedly shortens the lifetime of the anisotropic species generated by near-UV radiation at 77 K.

The ESR spectrum observed for Wd (Figure 1c) was composed of at least two paramagnetic species, i.e., it was composed of the anisotropic species with rhombic symmetry, a doublet splitting in the g_2 component (hereafter referred as species I), and an anisotropic species with small g-separation (hereafter referred as species II). When the microwave power for ESR excitation was increased to 5.0 mW, only the ESR spectrum of species I was recorded, as shown in Figure 1e. The g- and A-values of species I were evaluated to be $g_1 = 2.060$, $g_2 = 2.026$, $g_3 = 2.00$, and $A_2 = 0.8$ (mT), respectively.[9] The ESR parameters show excellent agreement with the disulfide neutral radical observed for the α-keratin of human finger nails ($g_1 = 2.061$, $g_1 = 2.025$, $g_1 = 2.000$, $A_2 = 0.8$ mT).[10] The structure of the radical has been alternatively formulated as $RSS^{\bullet}$ and sometimes as $RSSR'R^{\bullet}$. The most probable structure of species I is formulated as $RS_1S_2HR^{\bullet}$, on the basis of the results of ESR study conducted for several alkyl disulfide derivatives.[11] The relatively large g-separation of species I was attributed to the asymmetric structure of the disulfide moiety, in which the unpaired electron mainly occupied the σ^* orbital of the S_1 atom.

On the other hand, accurate g- and A-values of species II were still not obtained because of poor spectrum resolution. At present, the g-value of species II was approximately evaluated to be 2.005, at the intersection of the ESR line and base line (Figure 1c). The g-anisotropy of species II was apparently less than that of species I, suggesting that the disulfide bond of the radical had a symmetric structure. In fact, the line shape and g-value of species II were analogous to the anion radicals of disulfide group observed for α-keratin of human finger nail and for powder of lysozym.[10,12] On the basis of the ESR properties, species II was classified into the anion radical of the disulfide group of α-keratin ($RSSR^{-\bullet}$). The results of ESR measurements showed that when completely dried human hair was exposed to near-UV radiation, both anion radical ($RSSR^{-\bullet}$) and the neutral radical ($RSSHR^{\bullet}$) derived from the disulfide bond of α-keratin were formed.

The results of fast pulse radiolysis clarified that the disulfide radical reversibly dissociated to thiyl radical ($RS^{\bullet}$) and thiolate anion (RS^{-}) or thiol (RSH), as expressed in eq 1.[13] Furthermore, the presence of a proton source caused the equilibrium to shift to the right-hand side. The generated thiyl radical is readily changed to diamagnetic specie, such as disulfide formed by dimerization. The thiyl radical may become the source of the ESR signal, but the lifetime of the radical was too short to be detected by ESR measurements at 77 K.

$$RSSHR^{\bullet} \leftrightarrow RSH + RS^{\bullet} \rightarrow \text{diamagnetic species} \quad (1)$$

Consequently, the residual water present in the matrix of human hair accelerated the decomposition of disulfide radicals derived from α-keratin. The present findings indicate that dried white human hair can be considered a suitable sample for investigating the mechanisms of the radical reaction of the disulfide radicals in the matrix of human hair. Further investigations on exploring this possibility are now in progress with the aid of ESR measurements at 10 K.

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