ELECTRON PARAMAGNETIC RESONANCE STUDIES ON THE SELENIUM-REPLACED DERIVATIVES OF ADRENODOXIN: THE PRESENCE OF THE ONE SELENIUM-ONE SULFUR COMPOUND *

Kazuo Mukai**, John J. Huang***, and Tokuji Kimura
Department of Chemistry, Wayne State University
Detroit, Michigan 48202

Received September 21,1972; revised November 6,1972

Summary: We have replaced labile sulfur atoms of adrenodoxin with Se^{80}, or Se^{77} in the presence and absence of the labile sulfur source (Na_2S). When the labile sulfur source was absent but the selenium source was present in the reaction mixture for the reconstitution from apoadrenodoxin, the electron paramagnetic resonance spectra of the Se^{80} - Se^{80} and Se^{77}-Se^{77} derivatives had $g_{//} = 2.051$ and $g_{\perp} = 1.975$, and a distinct triplet of the $g_{//}$-signal, respectively. When both labile sulfur and selenium sources were present, new spectral species were observed. In the case of the Se^{80} - S^{32} derivative, the signal had $g_{//} = 2.038$, $g_2 = 1.973$, and $g_3 = 1.934$, and in the case of Se^{77} - S^{32} derivative, the $g_{//}$-signal was split into a doublet.

From these results, it is concluded that the compound with one sulfur and one selenium atom in the replacement of the two labile sulfur atoms of native adrenodoxin has a $g_{//}$-value of 2.038, and its paramagnetic center is more rhombohedral than those of native adrenodoxin and the Se - Se derivative.

Adrenodoxin is the iron-sulfur protein of a system which hydroxylates steroids in adrenal cortex mitochondria (1, 2). The detailed studies of this protein have extensively been carried out in this laboratory (3). Adrenodoxin contains in a molecular weight of 12,500, two iron atoms and two labile sulfur atoms, and undergoes a one-electron oxidation-reduction with an E'_o of -270 mV (J. J. Huang and T. Kimura, unpublished data).

In 1968, Tsibris et al. (4) reported the preparation and the electron paramagnetic resonance (EPR) spectrum of the Se^{80}-replaced putidaredoxin. Later, they extended the study, showing the hyperfine splitting pattern of the EPR signal of the Se^{77}-replaced derivative of putidaredoxin and adrenodoxin (5). Detailed studies of the selenium derivative of parsley ferredoxin have also been reported by Fee and Palmer (6).

To our knowledge, there is, however, no report concerning the Se-S mixed derivative of iron-sulfur proteins. We wish to report that the EPR signal with

---

* This study was supported by a Research Grant from the National Institutes of Health (AM-12713).
** The present address is Ehime University, Matsuyama, Japan
*** The present address is Washington University, St. Louis, Missouri

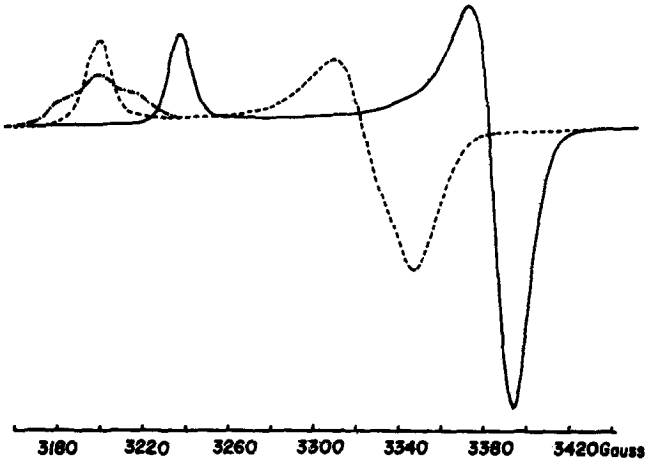

Figure 1. EPR Spectra of Native Adrenodoxin and its Se^{80} - Se^{80} and Se^{77} - Se^{77} Derivatives

A. _____ : native adrenodoxin; B, - - - - - - - - - : Se^{80} - Se^{80} derivative; C. _·_·_·_·_ : Se^{77} - Se^{77} derivative

The concentration of the protein samples was 2 mM as iron. Conditions of EPR spectroscopy: microwave power, 5 mWatt; frequency, 9.181 GH_z; modulation amplitude, 2.5 gauss; scanning rate, 50 gauss per minute; time constant, 1 second; temperature, 77°K.

$g_{//}$ = 2.038 is assigned to the derivative, where one of the two labile sulfur atoms of native adrenodoxin is replaced with one selenium atom.

Materials and Methods: Adrenodoxin was prepared from beef adrenal glands by the method described elsewhere (3). The ratio of the absorbance at 414 nm to that at 276 nm was 0.86. The apoprotein was prepared by repeated precipitation with 5% trichloroacetic acid solution until the labile sulfur content became negligible. The preparations of the selenium derivatives were carried out by a method similar to that described by Fee and Palmer (6). The selenium and sulfur mixed derivatives were prepared by adding both selenium (H_2SeO_3) and sulfur (Na_2S) compounds to the reaction mixture for the reconstitution. The reduction of the derivatives was carried out by the addition of solid dithionite to sealed anaerobic EPR sample tubes. EPR spectroscopy was carried out by the use of a Varian E-4 spectrometer at liquid nitrogen temperature, and optical absorption spectrophotometry was performed by the use of a Hitachi spectrophotometer (Model-124). The analyses of iron and labile sulfur were carried out by the methods described previously (2).

Results: Fig. 1, curves A, B, and C illustrate the EPR spectra of native adrenodoxin (S^{32} - S^{32}), the Se^{80} - Se^{80}, and Se^{77} - Se^{77} derivatives, respectively

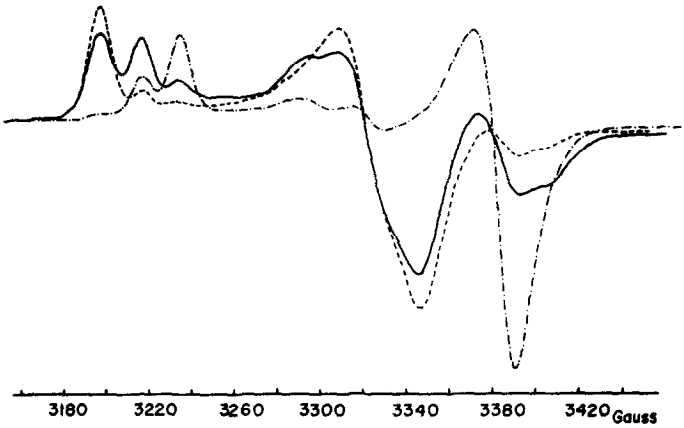

Figure 2. EPR Spectra of the Se^{80} - S^{32} Derivatives

A. ---------- : Se^{80} - S^{32} sample (1 : 2); B. _____ : Se^{80} - S^{32} sample (1 : 3)

C. _.-.-.-.-. : Se^{80} - S^{32} sample (1 : 5)

The concentration of the protein samples was 2 mM as iron. Conditions of EPR spectroscopy were the same as in Figure 1. The ratios in parentheses indicate those of selenium to sulfur content in the reaction mixture for the reconstitution.

The position of the $g_{//}$ -signal of the Se^{80} - Se^{80} derivative was shifted to a lower magnetic field, compared with that of native adrenodoxin, and the $g_{//}$ -signal of the Se^{77} - Se^{77} derivative was split into a triplet due to the nuclear spin of Se^{77}. It is noted here that the spectra of the Se-derivatives do not show any signal of impurity, such as native adrenodoxin. The results are essentially in agreement with those of adrenodoxin and putidaredoxin reported by others (4, 5).

Fig. 2, curves A, B and C show the overlapping spectra of the Se^{80} - S^{32} mixed derivatives of adrenodoxin with native adrenodoxin and its Se^{80} -Se^{80} derivative. The intensities vary depending on the ratios of S^{32} to Se^{80} in the reaction mixtures for the reconstitution.

Fig. 3 shows the EPR spectra of the Se^{77} - S^{32} derivatives with various ratios of Se^{77} to S^{32}. The curve A is the Se^{80} - S^{32} derivative, which is run for reference, and the curves B and C display the hyperfine splitting effected for the nuclear spin of Se^{77}. The g-values and the hyperfine tensor values for the z-axis directions ($A_{//}$) of these derivatives are summarized in Table I.

Discussion: In comparison with the spectra of the S^{32} - S^{32}, and Se^{80} - Se^{80} derivatives (Fig. 1, curves A and B), it was suggested to us that the signal at $g_{//}$= 2.038 (Fig. 2, curves A, B and C) would be due to the Se-S mixed derivative, where one of two labile sulfur atoms in native adrenodoxin is replaced with one

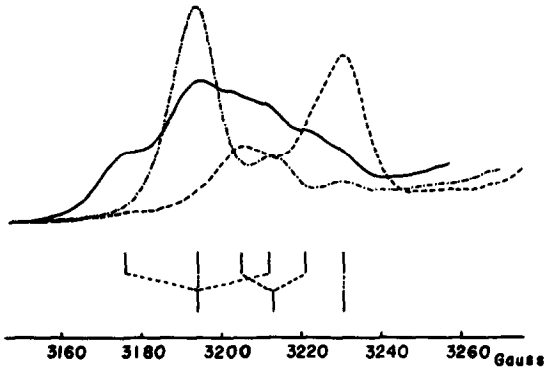

Figure 3. EPR Spectrum of the Se^{77} - S^{32} Derivative in Comparison with the Se^{80} - S^{32} Derivative

A. _·_·_·_·_ : Se^{80} - S^{32} sample (1 : 2); B. _____ : Se^{77} - S^{32} sample (1 : 3)

C. --------- : Se^{77} - S^{32} sample (1 : 5)

The concentration of the protein samples was 2 mM as iron. Conditions of EPR spectroscopy were the same as those in Figure 1, except the scanning rate was 25 gauss per minute.

selenium atom. This finding was confirmed by the use of the Se^{77}-derivatives.

When Se^{77} (I= 1/2) and S^{32} (I = 0) were used for the substitution of the labile sulfur in native adrenodoxin, the total number of the hyperfine splitting lines observed was six (Fig. 3, curves B and C). The number of the hyperfine splitting lines due to the Se^{77} - Se^{77} derivative is, by theory, three, of which the theoretical intensity ratio is 1 : 2 : 1 (5). The number due to the Se^{77} - S^{32} derivative must be two, of which the intensity ratio is 1 : 1. An additional signal at $g_{//}$ = 2.027 is due to the native protein with S^{32}, which does not show any splitting. Thus, the number of the hyperfine splitting lines observed can be accounted for by the sum of the numbers of the lines due to the Se^{77} - Se^{77}, Se^{77} - S^{32}, and S^{32} - S^{32} derivatives. Furthermore, the number, position, and the value of the hyperfine tensor along z-axis of the Se^{77} - S^{32} derivative are all reasonable, compared with those of the pure Se^{77} - Se^{77} derivative. In conclusion, the signal at $g_{//}$ = 2.038 is assigned to the Se-S derivative of adrenodoxin. From our present results, the previously published spectra of the Se -Se derivatives of iron-sulfur proteins (4, 5) appear to posses some trace amounts of the Se - S and S - S derivatives.

Compared with the axially symmetrical signal of native adrenodoxin, the $g_{\perp}$-signal of the pure Se^{80} - Se^{80} derivative is broad and has a small but significant shoulder, as shown in Fig. 1, curve B. The Se^{80} - S^{32} derivative exhibits the separate signals with different $g_{//}$, g_2, and g_3 -values, as listed

TABLE I. List of g-Values and Hyperfine Tensor Values

Derivatives	$g_{//}$	$g_{\perp}$	A//, gauss
$2Fe^{56}$ - $2S^{32}$	2.027 ± 0.002	1.940 ± 0.002	0
$2Fe^{56}$ - $2Se^{80}$	2.051 ± 0.002	1.975 ± 0.002	0
$2Fe^{56}$ - $2Se^{77}$	2.051 ± 0.002	1.974 ± 0.002	16 ± 1
$2Fe^{56}$ - Se^{80} - S^{32}	2.038 ± 0.002	g_2 = 1.973 g_3 = 1.934	0
$2Fe^{56}$ - Se^{77} - S^{32}	2.038 ± 0.002	g_2 = 1.973 g_3 = 1.934	16 ± 2

Since the EPR spectra of the Se-S derivatives were overlapping with those of the Se-Se derivative and native adrenodoxin, and the isolation of the Se-S derivative was impossible at present, the g-values of the Se-S derivatives were obtained by the following ways. The g3-values were calculated from the samples containing a high amount of the Se-S derivative and a low amount of the S-S derivative, where the contribution of the signal due to the Se-Se derivative was negligible (for instance, such a sample as shown in Figure 2, curve B). The g_2-values were calculated from the samples containing a high amount of the Se-S derivative and a low amount of the Se-Se derivative, where the contribution of the signal due to native adrenodoxin was very small (for example, such a sample as shown in Figure 2, curve C).

in Table I. In this context, it is concluded that the iron center of the Se-Se derivative slightly deviates from axial symmetry as seen in native adrenodoxin. It is also concluded that the Se-S derivative is most rhombohedral compared with the Se-Se and S-S derivatives. This suggests that the distortion of the paramagnetic center occurs to the greatest extent when one of two sulfur atoms in native adrenodoxin is replaced with a selenium atom. In other words, the selenium atom in the iron center is not positioned in exactly the same manner as the sulfur atoms in native adrenodoxin, although the Se-derivatives are enzymatically active (unpublished data).

Of interest here is the fact that the Se-S derivative of adrenodoxin has a similar symmetry of paramagnetic center to native spinach ferredoxin (g_x = 1.89, g_y = 1.96, and g_z = 2.04) and to the Se-Se derivative of putidaredoxin (g_x = 1.93, g_y = 1.98, and g_z = 2.04). Since the iron chromophore of these iron proteins consists of four cysteinyl sulfur atoms, two labile sulfur atoms, and two iron atoms, this difference must be explained by the difference in spatial

environment of the iron-sulfur chromophore of these proteins. The environment results in the distortion from axial symmetry in the case of spinach ferredoxin without the replacement of labile sulfur with selenium atom. In the case of putidaredoxin upon replacement of two labile sulfur atoms by two selenium atoms, and in the case of adrenodoxin upon replacement of one of the two labile sulfur atoms by one selenium atom, distortion also results. Furthermore, upon replacement of two labile sulfur atoms with two selenium atoms, parsley ferredoxin increases its degree of symmetry from rhombic to axial symmetry, a shift in a direction opposite to that observed in the case of adrenodoxin.

REFERENCES

1. Suzuki, K., and Kimura, T., Biochem. Biophys. Res. Commun., 19, 340 (1965)

2. Kimura, T., and Suzuki, K., J. Biol. Chem., 242, 485 (1967)

3. Kimura, T., in "Structure and Bonding", vol. 5, edited by C. K. Jørgensen, J. B. Neilands, R. S. Nyholm, D. Reinen, and R. J. P. Williams, Springer-Verlag, Berlin, 1968, p. 1.

4. Tsibris, J. C. M., Namtvedt, M. J., and Gunsalus, I. C., Biochem. Biophys. Res. Commun., 30, 323 (1968)

5. Orme-Johnson, W. H., Hansen, R. E., Beinert, H., Tsibris, J. C. M., Bartholomaus, R. C., and Gunsalus, I. C., Proc. Natl. Acad. Sci. U. S., 60, 368 (1968)

6. Fee, J. A. and Palmer, G., Biochim. Biophys. Acta, 245, 175 (1971)