RESONANCE RAMAN SCATTERING AND OPTICAL ABSORPTION OF ADRENODOXIN AND SELENA-ADRENODOXIN*

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Summary: Raman spectra have been recorded for native and selenium substituted adrenodoxin in dilute solution. Adrenodoxin shows three bands at 397, 350 and 297 cm^{-1}, all polarized, which can be associated with the iron-sulfur core. Selenium substitution leaves the 350 cm^{-1} band essentially unshifted, but the other two bands disappear and are replaced by new bands at 355 and 263 cm^{-1}. The 350 cm^{-1} band is assigned to stretching of iron-sulfur (cysteine) bonds, while the 397 and 297 cm^{-1} bands are associated with vibrations of the labile sulfur atoms. The iron-selenium charge transfer bands were observed at 438 and 480 nm for the oxidized form and at 580 nm for the reduced form. The reduced selena-adrenodoxin displayed absorption maxima at 4,450 and 5,550 cm^{-1}, which can be assigned to the d-d transitions of high-spin ferrous ion. From this data and the reported g-values of electron paramagnetic resonance signals, the spin-orbit coupling constants were calculated to be 170 and 210 cm^{-1} for the respective d-d transitions.

The potential of resonance Raman spectroscopy in probing biological chromophores in their usual high dilution has been demonstrated for rubredoxin (1), conjugated polyenes (2), heme proteins (3), and vitamin B_{12} (4). In this technique molecular vibrations associated with the chromophore exhibit enhanced Raman scattering when the exciting radiation falls within an electronic absorption band. For adrenodoxin excitation in the visible Fe-S charge transfer bands (414 and 455 nm) (5) should enhance Raman bands

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Abbreviations used: S*, labile sulfur atom; EPR, electron paramagnetic resonance.

associated with the iron-sulfur core. The spectrum of rubredoxin demonstrates that this is indeed the case (1).

While x-ray crystal structures are in hand for examples of 1Fe-0S* (rubredoxin) (6), 4Fe-4S* (HiPIP) (7), and 8Fe-8S* (Peptococcus aerogenes ferredoxin) (8) proteins, no structure is yet available for 2Fe-2S* proteins. They have been extensively characterized by a variety of physical techniques (9), however. It is well established that the iron and labile sulfur atoms, as well as several cysteine sulfur atoms are in intimate contact. The reduced proteins contain high-spin Fe(II) antiferromagnetically coupled to its high-spin Fe(III) partner. The oxidized form contains two antiferromagnetically coupled Fe(III) atoms (10). The oxidation state of the labile sulfur atoms remains uncertain, both sulfide (11) and disulfide (12) being candidates. Here we report Raman results on adrenodoxin, a representative of the interesting class of 2Fe-2S* proteins, and on its selenium substituted analog. Optical absorption characteristics for selena-adrenodoxin are also reported.

Materials and Methods: Adrenodoxin was prepared from beef adrenal glands by the method described elsewhere (5). 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 following a Sephadex G-25 gel filtration. The labile sulfur content of the resulted apoprotein was negligible. The preparations of the selenium derivatives (13) was carried out by a method similar to that described by Fee and Palmer (16). As in other 2Fe-2S* proteins (14-17) Se substitution gives a biologically active molecule (13).

The Raman spectrometer was equipped with a Coherent Radiation 52G Ar^+ laser, a Spex 1401 double monochromator and a cooled ITT FW-130 phototube. Spectra were obtained in 1 mm diameter glass capillary tubes using transverse laser (4880 Å) excitation. A polaroid disk was used to analyze the scattered light.

Optical absorption spectra were recorded with a Hitachi spectrophoto-

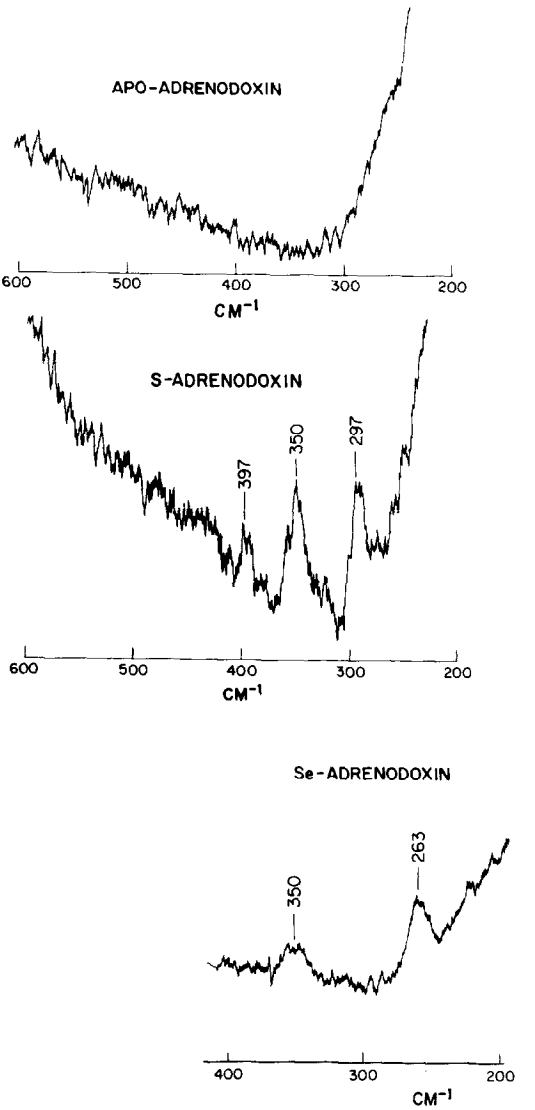

Figure 1. Resonance Raman Spectra of Apo-adrenodoxin, Adrenodoxin, and Selena-adrenodoxin

Protein concentrations were about 1 mM (pH = 7). The spectra were obtained in 1 mm diameter glass capillary tubes with transverse excitation by a 4880 Å laser beam (100-400 mw). Slit width: 10 cm^{-1}, scan speed: 30 cm^{-1} per minute, and time constant: 10 seconds. In the spectrum of selena-adrenodoxin, the scan speed is 20 cm^{-1} per minute.

meter (model EPS-3). For the near infrared absorption spectrum a PbS detector was used. A sample of the selenium derivative was subjected to deuterium exchange by repeating lyophilization and dissolving in D_2O. The

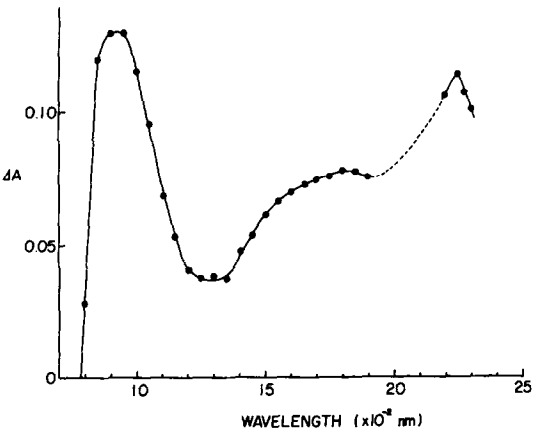

Figure 2. Difference Spectrum Between Reduced and Oxidized Selena-adrenodoxin in the Near Infrared Region

The protein concentration was 1.3×10^{-3} M in a D_2O solution containing 0.01 M Tris buffer (pH 7.4). Due to the presence of trace H_2O in the sample, the absorbance between 1,900 and 2,200 cm^{-1} (dotted line) could not be reliably measured. The reduced form was made by adding solid dithionite in the presence of a catalytic amount of methylviologen under anaerobic conditions.

The theoretical background for the calculation of spin-orbit coupling constants and the assignment of the d-d transition bands were discussed in a previous paper (10).

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near infrared absorbance was measured by taking the difference between the reduced and oxidized spectra.

Results and Discussion: Figure 2 shows spectra obtained in (10^{-3} M) solutions of oxidized S-adrenodoxin, oxidized Se-adrenodoxin and apoadrenodoxin, obtained with 4880 Å Ar^+ laser excitation. The background scattering from the polypeptide is quite high, but there are no discrete Raman bands. Three Raman bands are reproducibly observed for native adrenodoxin at 397, 350, and 297 cm^{-1}. All three are polarized and therefore arise from totally symmetric vibrations. When selenium is substituted for labile sulfur, the 350 cm^{-1} band remains but appears as a double peak, with a separation of about 10 cm^{-1}, while the other two disappear, and a new band is observed at 263 cm^{-1}.

The 350 cm^{-1} band can therefore be assigned with some confidence to Fe-S (cysteine) bond stretching. This frequency accords well with the Fe-S

(cysteine) vibrations observed in rubredoxin at 314 and 368 cm^{-1} (1). We note that the far infrared spectrum of native adrenodoxin (18) shows a single broad band at 344 cm^{-1}. The 297 and 397 cm^{-1} Raman bands, which are absent in the Se-adrenodoxin spectrum, are clearly associated with vibrations of the labile sulfur atoms. The corresponding vibrations are expected at lower frequencies when the heavier selenium atoms are substituted, and may tentatively be identified with the 263 cm^{-1} band and with the second peak in the 350 cm^{-1} envelope ($\sim$355 cm^{-1}) of the Se-adrenodoxin spectrum. The frequency lowering would then be about 40 cm^{-1} for both bands. Detailed descriptions of the associated vibrations cannot presently be given. Normal coordinate calculations on model structures are currently in progress.

The UV-visible absorption spectrum of oxidized Se-adrenodoxin has peaks at 258, 264, 335, 438 and 480 nm, with shoulders at 275 and 280 nm. The dithionite-reduced protein shows maxima at 310, 350 and 580 nm, with shoulders at 400 and 470 nm. Replacement of labile sulfur with selenium is accompanied by a pronounced red shift (about 25 nm) of the visible absorption bands. The near-infrared absorption spectrum of reduced Se-adrenodoxin (Figure 2) shows bands at 4,550 and 5,550 cm^{-1}, as expected for the d-d transitions of high-spin Fe(II) (10). Their molar extinction coefficients, 80 and 50 $cm^{-1}\ \underline{M}^{-1}$ respectively, are similar to those of the corresponding bands in the native protein (10). From the optical frequencies and the EPR g-values (19) ($g_{//} = 2.051$, $g_{\perp} = 1.975$) spin-orbit coupling constants were estimated. The calculated values, $\zeta_x = 170\ cm^{-1}$ and $\zeta_y = 210\ cm^{-1}$ are somewhat less than those of native adrenodoxin (10).

References

1. Long, T. V., and Loehr, T. M., (1970), J. Amer. Chem. Soc., 92: 6384.
2. a) Rimai, L., Gill, D., and Parsons, J., (1971), J. Amer. Chem. Soc., 93:1353. b) Gill, D., Kilponen, R. G., and Rimai, L., (1970), Nature, 227:743.
3. Spiro, T. G., and Strekas, T. C., (1972), Proc. Nat. Acad. Sci. U. S., 69:2624.
4. Wozniak, W. T., and Spiro, T. G., (1973), J. Amer. Chem. Soc., 95:3402.
5. Kimura, T., (1968), Structure and Bonding, 5:1.

6. Herriot, J. R., Sieker, L. C., Jensen, L. H., and Lovenberg, W., (1970), J. Mol. Biol., 50:391.
7. Carter, C. W., Jr., Freer, S. T., Xuong, Ng. H., Alden, R. A., and Kraut, T., (1971), Cold Spring Harbor Symp. Quant. Biol., 36:381.
8. Sieker, L. C., Adman, E., and Jensen, L. H., (1972), Nature, 235:40.
9. Tsibris, J. C. M., and Woody, R. W., (1970), Coordination Chemistry Reviews, 5:417.
10. Eaton, W. A., Palmer, G., Fee, J. A., Kimura, T., and Lovenberg, W., (1971), Proc. Nat. Acad. Sci. U. S., 68:3015.
11. Blomstrom, D. C., Knight, E., Jr., Phillips, W. D., and Weiher, J. F., (1964), Proc. Nat. Acad. Sci. U. S., 51:1085.
12. Kimura, T., Nagata, Y., and Tsurugi, J., (1971), J. Biol. Chem., 246:5140
13. Mukai, K., Huang, J. J., and Kimura, T., to be published.
14. Tsibris, J. C. M., Namtvedt, M. J., and Gunsalus, I. C., (1968), Biochem. Biophys. Res. Commun., 30:323.
15. Orme-Johnson, W. H., Hansen, R. E., Beinert, H., Tsibris, J. C. M., Bartholomaus, R. C., and Gunsalus, I. C., (1968), Proc. Nat. Acad. Sci. U. S., 60:368.
16. Fee, J. A., and Palmer, G., (1971), Biochim. Biophys. Acta, 245:175.
17. Fee, J. A., Mayhew, S. G., and Palmer, G., (1971), Biochim. Biophys. Acta, 245:196.
18. Kimura, T., and Huang, J. J., (1970), Arch. Biochem. Biophys., 137:357.
19. Mukai, K., Huang, J. J., and Kimura, T., (1973), Biochem. Biophys. Res. Commun., 50:105.