SELENIUM PROTEINS IN OVINE TISSUES II. SPECTRAL PROPERTIES OF A 10,000 MOLECULAR WEIGHT SELENIUM PROTEIN.*

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SUMMARY

A selenium-containing protein of 10,000 molecular weight, which is absent in muscle of selenium deficient lambs, was purified from the muscle extract of lambs injected with selenium. The absorption, circular dichroic, and magnetic circular dichroic spectra of the protein with and without dithionite markedly resemble the oxidized and reduced spectra reported for cytochrome C. Thus, this protein contains a heme group identical to cytochrome C, and may be a selenium containing cytochrome.

Although white muscle disease (WMD) was shown to be a selenium responsive myopathy about 15 years ago (1), the metabolic functions of this element in preventing this disorder have not been established. In the study of the distribution of selenium between different molecular weight proteins in ovine tissues, a selenium containing protein of about 10,000 molecular weight was found to be absent in muscle and heart of WMD lambs, but present in normal (Se injected) lambs (2). Evidence has been presented to indicate that selenium is incorporated into as well as required for the formation of this protein (3). Therefore, this protein was purified and characterized from muscle of selenium injected lambs with the idea that its characteristics might reveal some information concerning the metabolic roles of selenium. This report deals with its spectral properties, indicating the presence of a heme group as part of the molecule.

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MATERIALS AND METHODS

The 10,000 molecular weight selenium containing protein was isolated from an extract of 2 week-old lamb muscle by ammonium sulfate fractionation, gel filtration chromatography (Biogel P-60), and hydroxylapatite chromatography (3). The purity of the protein, based on disc gel electrophoresis, was over 90%. The spectral studies were conducted on the protein (200 to 400 μg/ml) dissolved in 0.05 M phosphate buffer, pH 7.2.

The absorption spectra were measured at 25° with a Cary Model 15 spectrophotometer equipped with a 0 to 1.0 slidewire. Magnetic circular dichroic (MCD) and circular dichroic (CD) spectra were obtained at 25° with a Cary Model 61 spectropolarimeter with or without a magnetic field of about 40 Kgauss in cells of 0.5 to 1.0 cm light path. MCD and CD spectra are additive; hence the MCD spectra have been corrected for CD component. Molar ellipticity, $[\theta]_\lambda$, is given in degree cm^2 $dmole^{-1}$, and molar magnetic ellipticity, $[\theta]_m$, is given in degree cm^2 $dmole^{-1}$ $Kgauss^{-1}$.

RESULTS

As indicated in figure 1, the protein exhibits an absorption maxima at 410 nm. However, the addition of dithionite shifts this peak to 415 nm, with the production of two additional peaks at 520 and 550 nm. This is very similar to that reported for the oxidized and reduced spectra for cytochrome C (4,5).

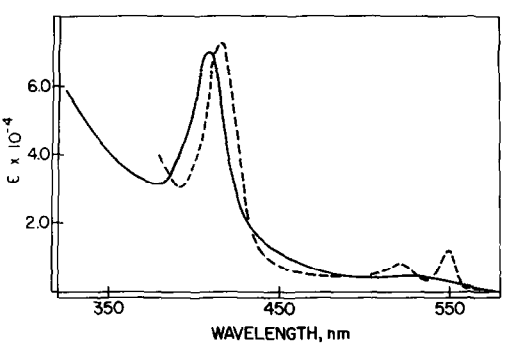
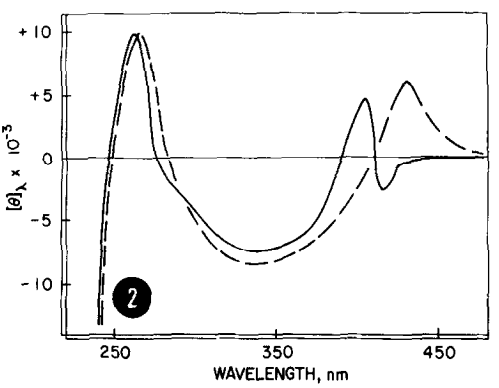


Figure 1. Absorption Spectra of the Selenium Containing Protein with (-----) and without (———) Dithionite.

Examination of the CD spectra (figure 2) revealed a negative band at 415 nm and a negative trough between 380 and 280 nm. Positive bands were found at 405 and at about 260 nm. Addition of dithionite, however, abolished the negative band at 415 and shifted the positive one at 405 to 415 with only minor changes in the rest of the spectra. This shows a very similar resemblance to that seen for cytochrome C (6).

MCD spectra of this protein (figure 3) revealed a negative maximum at 418 nm with small negative bands at 570, 555 and 340 nm. A positive maximum



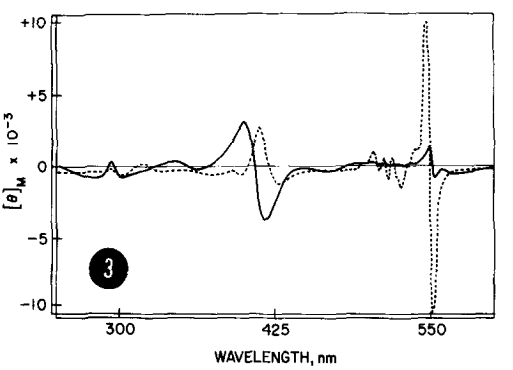


Figure 2. Circular Dichroic Spectra of the Selenium Containing Protein with (-----) and without (——) Dithionite.

Figure 3. Magnetic Circular Dichroic Spectra of the Selenium Containing Protein with (-----) and without (——) Dithionite.

is found at 405 with minor positive bands between 450 and 550 in the untreated protein. Addition of dithionite, however, resulted in a negative maximum at 550 and a positive maximum at 545. The positive maximum at 405 of the untreated protein was shifted to 415 and the negative maximum band at 418 was decreased and shifted to about 425 nm by addition of dithionite. Again, in agreement with the absorption and CD spectra, this is very markedly similar to that observed for the oxidized and reduced MCD spectra of cytochrome C (7).

DISCUSSION

Based on the spectral properties, the 10,000 molecular weight selenium containing protein from ovine muscle contains a heme chromophore whose spectral properties are identical to that bound to cytochrome C. Based on the extinction coefficient derived for reduced cytochrome C at 550 nm (5), this selenium protein was calculated to contain 1.2 moles of heme per 10,000 grams of protein, suggesting one heme group per mole protein.

The spectral properties of this protein are markedly similar to cytochrome C, even though the molecular weight and amino acid composition are dissimilar (3), resembling instead those of cytochrome b_5 (8,9). The spectral characteristics, however, differ from those reported for cytochrome b_5 (9). Thus, this selenium containing protein appears to possess the same protein composition as cytochrome b_5, but contains a heme group identical to that bound to cytochrome C.

If our protein is indeed a cytochrome, this is the first evidence that selenium may be part of as well as necessary for the formation of a cytochrome. Furthermore, since the cytochromes are isolated from the microsomal or mitochondrial fractions, the present work indicates that the heme proteins in the soluble fraction deserve extensive study, at least in muscle and heart. No discrete selenium containing proteins in the 10,000 molecular weight range were found in the soluble fraction of the liver (2). The essentiality of selenium for the formation of the heme proteins (cytochromes) in microsomes and mitochondria are presently under investigation in our laboratory. Based on its ability to catalyze the reduction of cytochrome C in the presence of glutathione, selenium was recently suggested to be involved in respiration (10). This along with our present results support the vague but persistent impressions that selenium is somehow important in oxidation-reduction reactions in many kinds of biological systems (11,12).

Strong evidence has been presented for the requirement of selenium as an integral part of the enzyme, glutathione peroxidase (13). The present report

suggests that in addition to this enzyme, selenium is also required for the formation of a hemoprotein, presumably a cytochrome.

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