Organic Solvent Effects on the Chromophore of Adrenal 2 Iron - 2 Labile Sulfur Protein (Adrenodoxin)

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Summary: The effects of organic solvents on the absorption spectrum of adrenodoxin were examined. The solvents produced hyperchromicity in the region between 250 nm and 700 nm. In dimethyl formamide, and dimethyl sulfoxide, a red shift of the principal peaks was observed. while in methanol, glycerol, and ethylene glycol, a slight blue shift was found. The resulting spectrum is somewhat similar to that of either chloroplast or clostridial ferredoxins, probably due to the environmental and/or symmetrical changes in the 2 iron - 2 labile sulfur chromophore.

The iron-sulfur linkage in ferredoxins has the following unusual characteristics (1,2,3): (a) the oxidized form of the iron has no electron paramagnetic resonance (EPR), while the reduced form of adrenodoxin (4) and putidaredoxin (5) exhibits an axial EPR signal at $g_{\perp} = 1.94$ and $g_{//} = 2$, and that of spinach (6) and clostridial (7) ferredoxins displays a rhombic signal at $g_x = 1.89$, $g_y = 1.94$, and $g_z = 2$, (b) it is likely at present that the diamagnetic oxidized protein has an extremely strong magnetic exchange coupling energy, if there is antiferromagnetic exchange coupling between iron atoms (8), and (c) in the reduced form, one electron added interacts with the 2 iron atoms (3).

Recently, Coffman and Stavens (9) reported a weak solvent perturbation of the native conformation of the 2 iron - 2 labile sulfur chromophore of spinach ferredoxin. They demonstrated that the EPR anisotropy changes were induced by small amounts of methanol and propanol. In this paper, we wish to report a remarkable stability and solubility of oxidized adrenodoxin in a variety of organic solvents and the induced changes in optical absorption of the 2 iron - 2 labile sulfur grouping caused by these solvents.

Materials and Methods

Adrenodoxin was prepared as described previously (1). $A_{414}/A_{276} = 0.79$ and 1.9 g atoms of iron were bound per mole of protein.

Optical absorption spectroscopy was carried out by the use of Hitachi spectrophotometers (EPS-3, and 124) at a controlled temperature.

Results

Oxidized adrenodoxin is very stable at high concentrations of organic solvents. At 25° for 60 minutes, decreases in absorbance at 414 nm were found to be 0, 3, 7, 7, 8, and 27% in 98.3% (v/v) glycerol, 80% acetone, 98.3% dimethyl formamide, 98.3% pyridine, 98.3% dimethyl sulfoxide, and 80% methanol, respectively. No turbidity was observed at these concentrations, but more than 80% methanol and acetone produced some turbidity. By p-hydroxymercuribenzoate titrations, it was revealed that in 80% methanol or 98.3% dimethyl formamide solution of adrenodoxin the number of the sulfhydryl groups participating in the chromophore is 4 (2 cysteine + 1 labile sulfur) per iron atom. This indicates that

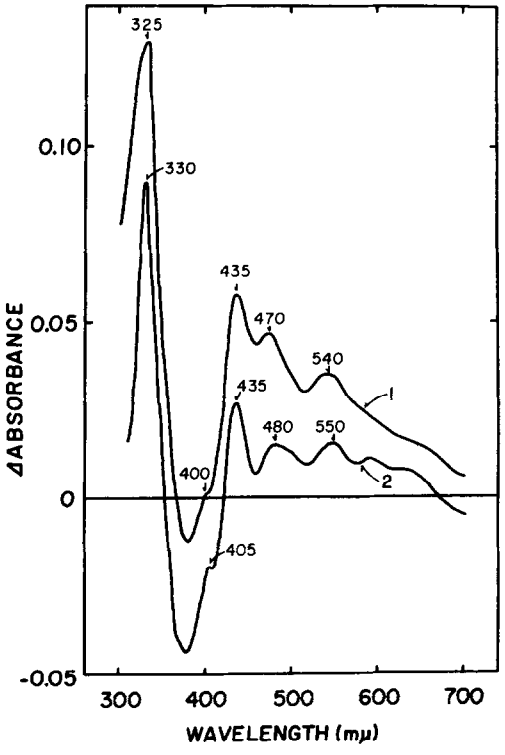

Fig. 1, Difference spectra of adrenodoxin with and without solvents of large dipole moment

Adrenodoxin concentration was 3.36×10^{-5} M. In curve 1, dimethyl formamide(DMF), 98.3%, and 0.01 M phosphate buffer (pH 7.4), 1.7%. In curve 2, dimethyl sulfoxide(DMS), 98.3%, and 0.01 M phosphate buffer (pH 7.4), 1.7%.
The spectra were taken at 30 sec, after mixing adrenodoxin with solvent at 25°. The controlled cuvette contained the same components as the experimental one, except the solvent. Dipole moments of solvents in the gas phase are as follow: DMF, 3.82; DMS, 3.96 debyes.

the components of the chromophore are not changed by the presence of solvents. Unlike the oxidized form, reduced adrenodoxin chromophore was found to be unstable at the high concentrations of organic solvents tested.

Marked increases in absorbance at 325-330 nm were most evident. The two main peaks (414, and 455 nm) and two shoulders (510, and 550 nm) in the oxidized protein (10) also exhibited hyperchromicity in the presence of organic solvents. As reported previously (1), the native protein in aqueous solution has a characteristic splitting at the region between 290 and 250 nm, presumably reflecting absorbances of the phenylalanine and tryosine residues (there is no tryptophan). 80% methanol and 98.3% ethylene glycol induced a distinct splitting of these peaks and an increase in absorbance in the aromatic region. In 80% methanol, 98.3% glycerol, and 98.3% ethylene glycol solutions, a blue shift of the main peaks occured, whereas in 98.3% dimethyl formamide, and

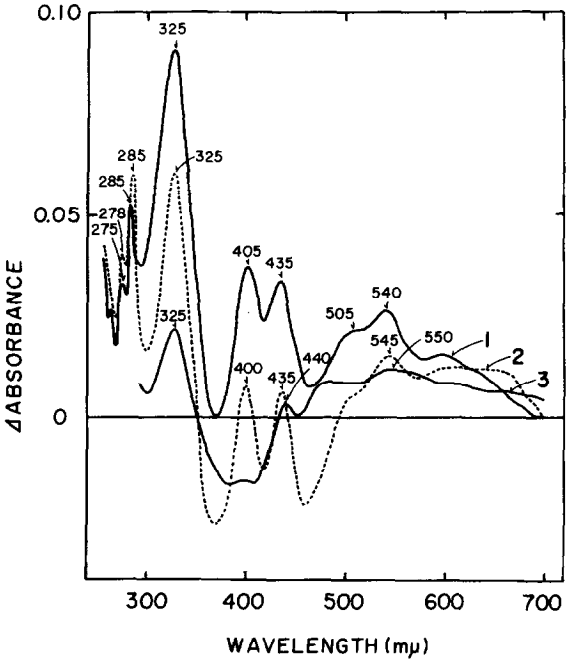

Fig. 2, Difference spectra of adrenodoxin with and without solvent of small dipole moment

Adrenodoxin concentration was 3.36×10^{-5} M. In curve 1, 80% methanol and 20% 0.01 M phosphate buffer (pH 7.4). In curve 2, and 3, 98.3% ethylene glycol or 98.3% glycerol and 1.7% 0.01 M phosphate buffer (pH 7.4). The other conditions are the same as in Fig. 1. Dipole moments of solvents are as follow: methanol, 1.70; ethylene glycol, 2.3; and water, 1.9 debyes.
In a 98.3% pyridine solution, a red shift was observed, although the dipole moment is lower than that of ethylene glycol.

98.3% dimethyl sulfoxide solutions a red shift was observed. These results are shown in Figures 1 and 2.

Changes in optical absorbances at 325 nm were measured in various concentrations of methanol in water. The results are plotted as the dielectric parameter vs. optical absorbance changes (Figure 3).

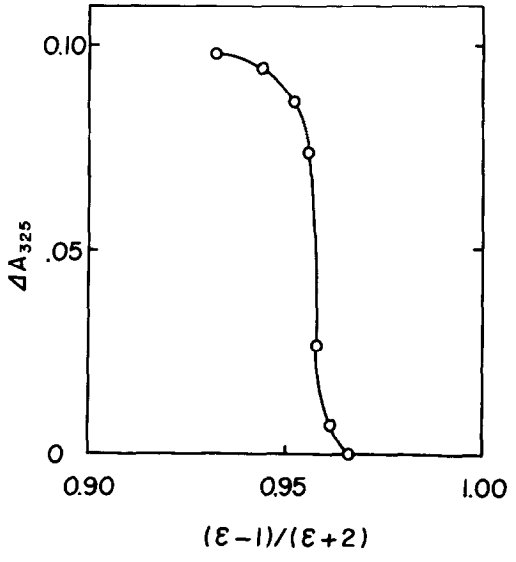

Fig. 3, Plots of dielectric parameters vs. optical absorbance changes due to aqueous methanol

ε represents dielectric constant which is calculated by intrapolation from values of water (80) and methanol (33).

Discussions

Adrenodoxin is a redox intermediate in NADPH-dependent steroid hydroxylase reactions in adrenal cortical mitochondria (11). The physical and chemical properties of this protein have been studied extensively (1). The amino acid sequence of adrenodoxin has recently been reported by Tanaka et al. (12), indicating a marked difference of adrenodoxin from chloroplast ferredoxins in agreement with our previous implication that there is a considerable difference in protein conformations between adrenodoxin and spinach ferredoxin (13). Yet, it has suggested that the iron-labile sulfur chromophore is basically the same among ferredoxins (14). The differences in protein conformations and amino acid sequences between ferredoxins, however, would secondarily affect the common iron-sulfur linkage in order to produce slightly different physical properties such as optical absorption spectra, optical activities and EPR signals. In fact, the symmetry around the

iron-sulfur linkage of adrenodoxin in water is axial while that of spinach and clostridial ferredoxins is rhombic. Furthermore, the environment of the chromophore in adrenodoxin and spinach ferredoxin are somewhat different with respect to their interacting aromatic amino acids (14).

We have shown in this communication that the solvent perturbation causes either red or blue shifts of the absorption peaks of adrenodoxin. In a medium with a large dipole moment, the solvent-induced shift of the adrenodoxin chromophore (414, 455 nm) becomes closer to the spinach-type (420, 460 nm), while in a medium with a small dipole moment it is closer to the clostridial ferredoxin type (390 nm). Of great interest is the fact that organic solvents can change the optical properties of adrenodoxin to make it more like those of other ferredoxins. Coffman and Stavens (9) have shown that the methanol-induced line sharpening of EPR signal of spinach ferredoxin is of axial symmetry which is contrast to the rhombic symmetry in water. At present, however, the change of symmetry of the chromophore of adrenodoxin toward rhombicity by organic solvents can only be speculation.

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