PMR Characterization of Alfalfa and Soybean Ferredoxins: The Existence of Two Ferredoxins in Soybean

by

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Summary

Observation of contact-shifted resonances in the proton magnetic resonance spectra of the oxidized and reduced forms of alfalfa and soybean ferredoxins confirms previous conclusions that the two iron atoms of each of these plant ferredoxins are antiferromagnetically exchange coupled. The contact-shift spectrum of the reduced soybean preparation is best interpreted in terms of the presence of two distinct ferredoxins in approximately equal concentration. It is concluded that contact-shift spectra may be useful for studying the genetic controls of plant ferredoxins and for elucidating phylogenetic relationships of plants.

The physical and chemical properties of the iron-sulfur proteins associated with plants have been extensively characterized.[1] Their molecular weights are about 10,500, and a prominent structural feature, apparently common to all, is the presence at the redox center of two nonheme iron and two "inorganic" sulfur atoms.

As part of a general study of the electronic and geometrical structures of the iron-sulfur proteins by proton magnetic resonance (PMR)

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spectroscopy, we have observed contact-shifted resonances in both redox forms of the ferredoxins from spinach and parsley.[2] These resonances originate from interactions with paramagnetic centers present, apparently, in the oxidized as well as reduced forms of these proteins. The patterns of contact-shifted resonances observed in the spinach and parsley ferredoxins are closely related, but differ in detail. On the other hand, contact-shift spectra observed in the two-iron ferredoxins appeared qualitatively different from those encountered with the one-iron rubredoxin from Clostridium pasteurianum,[3] the four-iron high potential iron protein from Chromatium,[4] and the eight-iron ferredoxin from C. pasteurianum.[5] In this note the patterns of contact shifts in the PMR spectra of the two redox forms of alfalfa ferredoxin are shown to be very similar to those of the spinach and parsley ferredoxins. In contrast, while the contact-shift spectrum of ferredoxin from soybean (cultivar Kent) is obviously related tothose of the other plant ferredoxins, it is concluded that two distinctly different ferredoxins are present in the soybean preparation in approximately equal concentrations.

Experimental

Ferredoxins from alfalfa[6] and soybean (cultivar Kent) were isolated by the method of Keresztes-Nagy and Margoliash;[7] purification of the latter protein was completed by passage through a column (3.0 x 90 cm) of Sephadex G-75 (Pharmacia; fine) equilibrated against 0.15 M NaCl, 0.01 M Tris·HCl pH 7.8 buffer. Elution was accomplished with a linear gradient of 0.2 to 0.4 M NaCl, 0.01 M Tris·HCl pH 7.8. Protein concentrations were determined spectrophotometrically at 422 mμ, using an extinction coefficient of 0.79 cm liter/g.[7] Reduction and spectroscopic techniques similar to those employed with spinach and parsley ferredoxins[2] were used here. Dithionite reduced alfalfa and soybean ferredoxins both yielded the characteristic g = 1.94 electron spin resonance absorption associated with other plant ferredoxins.[8]

Results and Discussion

The 220 MHz PMR spectra of oxidized alfalfa ferredoxin at 6°, 15°, 23°, and 30°C. are presented in Figure 1. Resonance absorption

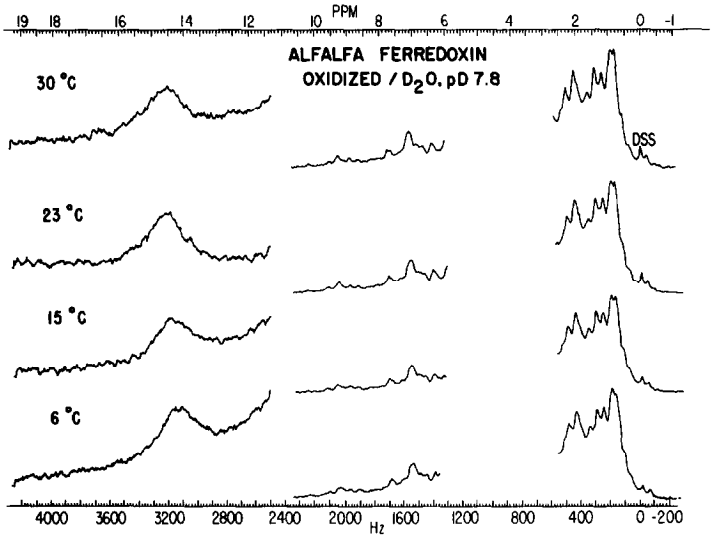

Figure 1. Temperature dependence of the 220 MHz PMR spectra of oxidized alfalfa ferredoxin. 10% (w/v) in D_2O at pD 7.8 (0.2 M Tris·DCl). The region of HDO absorption (3-5 ppm) has been omitted from the spectrum. The -1 to 10 ppm regions are derived from single spectral scans, while the 11 to 19 ppm regions represent 100 computer accumulations.

in the -1 to +8 ppm range is qualitatively similar to that observed for diamagnetic proteins, and will not concern us further here. Upon computer averaging, a broad resonance corresponding in intensity to a single proton emerges from the background in the +14 to +15 ppm region of resonance absorption. A similar resonance is observed in the oxidized forms of the ferredoxins from spinach and parsley and was attributed to a contact interaction from residual paramagnetism of two antiferromagnetically exchange coupled, high-spin ferric atoms.[2] The resonance was assigned to one of eight β-CH_2 protons of four cysteine residues thought to bind the iron-sulfur moiety to the polypeptide chain. The seven other expected but unresolved resonances of unit intensity for the oxidized spinach and parsley ferredoxins are believed to reside upfield and to be indistinguishable from the resonance absorption of the bulk of the protein. Analysis of the contact-shift region of resonance absorption of oxidized alfalfa

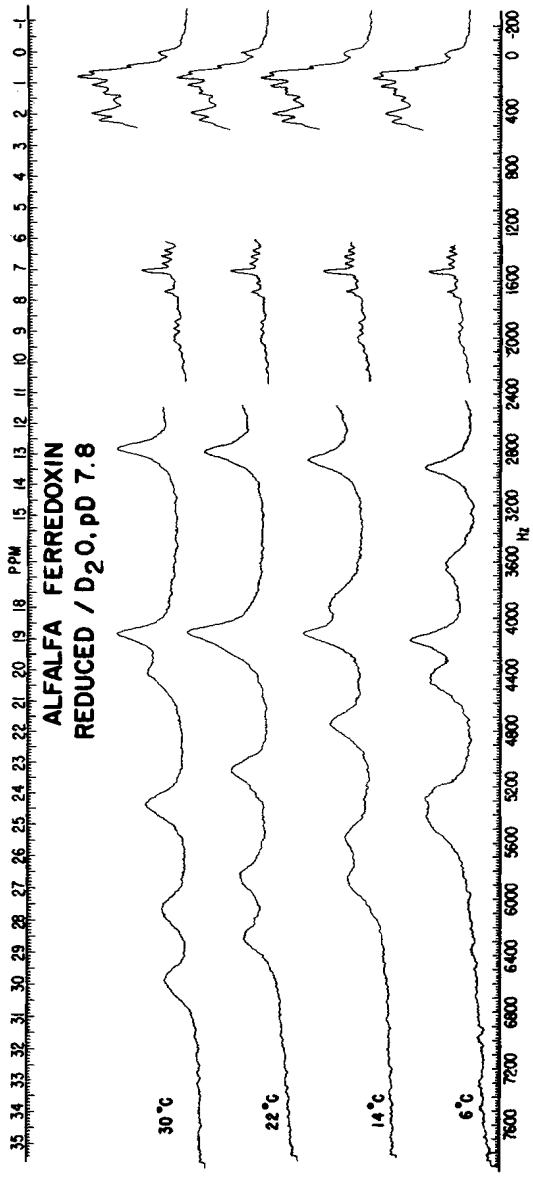

Figure 2. Temperature dependence of the 220 MHz PMR spectra of reduced alfalfa ferredoxin. 10% (w/v) in D_2O at pD 7.8 (0.2 M Tris·DCl). Spectral accumulations for the upfield and downfield regions were the same as for Figure 1. Reduction was accomplished with a 50% molar excess of solid sodium dithionite.

ferredoxin proceeds along identical lines. The temperature dependence of the low-field region of the PMR spectrum of alfalfa ferredoxin, including the contact-shifted resonance between 3,100 Hz (14.1 ppm) and 3,200 Hz (14.5 ppm), is plotted on the left-hand side of Figure 3.

The temperature dependence of the PMR spectrum of reduced alfalfa ferredoxin is given in Figure 2. The contact-shift region of the reduced ferredoxin, +12 to +31 ppm, is much richer than that of the oxidized form. The temperature dependences of the six resolved contact-shifted resonances of reduced alfalfa ferredoxin, each of intensity corresponding to one proton per molecule, are plotted on

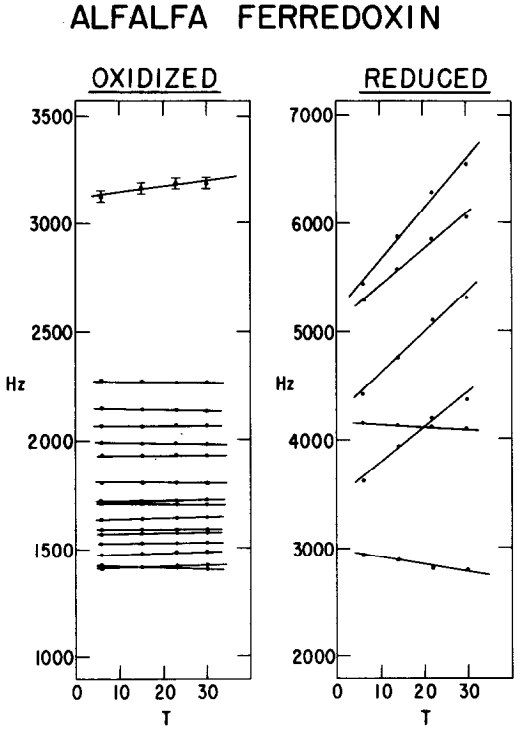

Figure 3. Temperature dependences of the lowfield resonances of oxidized (left) and reduced (right) alfalfa ferredoxin. Only the resonance that occurs between 3,100 and 3,200 Hz in oxidized alfalfa ferredoxin can be clearly identified with a contact-shift origin, whereas all of those displayed for reduced alfalfa ferredoxin appear subject to contact interaction.

the right-hand side of Figure 3. Again, based on the presumed structure, eight resonances corresponding to the β-CH_2 protons of four cysteine residues are expected. As for the oxidized form, it is suggested that the two missing resonances are located upfield in the region of resonance absorption of the carbon-bound protons of the rest of the protein.

Of the six resolved contact-shifted resonances of reduced alfalfa ferredoxin, four rapidly move downfield (increase contact-shift) with temperature and two slowly move upfield (decrease contact-shift) over the 6° to 30°C. range. For reduced spinach ferredoxin, six contact-shifted resonances were observed, four of which rapidly moved downfield with temperature and two very slowly moved upfield.[2] The six contact-shifted resonances of reduced spinach ferredoxin were qualitatively similar to the six of reduced alfalfa ferredoxin with respect to position and temperature dependence, but were readily distinguishable on quantitative comparison. Eight contact-shifted resonances, apparently a full complement, were observed for reduced parsley ferredoxin, four of which rapidly moved downfield with temperature and four of which moved upfield slowly.[2] For parsley ferredoxin the contact-shift spectrum of the reduced form was qualitatively as well as quantitatively different from those of the alfalfa and spinach ferredoxins.

We suggest that these rather striking differences in contact-shift spectra of the plant ferredoxins in the reduced form arise from variations in angles about the C_β-S bonds of the cysteine residues, such variations in turn being produced by differences in conformation of the polypeptide part of the protein. These conformational differences would be consequences of sequence variations among the polypeptides of the various plant ferredoxins.[9]

Rather subtle structural and compositional differences between the plant ferredoxins, thus, may be reflected in the contact-shift region of resonance absorption of the reduced forms. In support of this, the temperature dependence of the contact-shift region of resonance absorption of the reduced form of a ferredoxin preparation of soybeans (cultivar Kent) is presented in Figure 4. The striking feature is that instead of the six

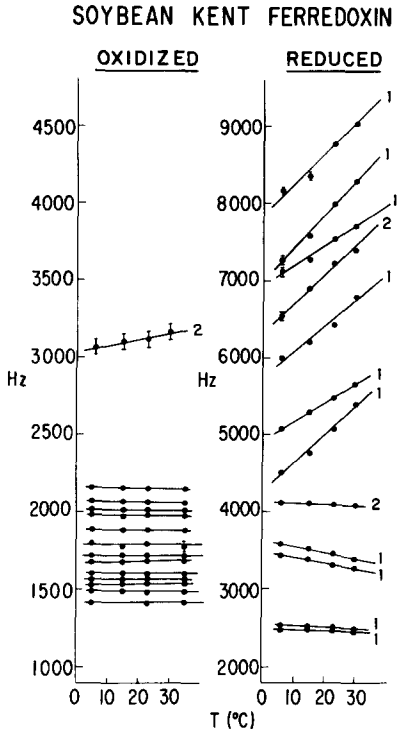

Figure 4. Temperature dependences of the lowfield resonances of oxidized (left) and reduced (right) soybean ferredoxin. Numbers of protons/ferredoxin molecule responsible for resonance intensities are indicated to the right of each resonance. The intensity data were treated on the basis of the presence of two alfalfa ferredoxins in equal concentration. Only those resonances with associated intensity indices are identified with a contact interaction origin.

or eight contact-shifted resonances observed for the reduced forms of the three other plant ferredoxins, fourteen resonances are actually detected for the reduced soybean ferredoxin.* Eight rapidly move to lowfield with temperature, and six slowly shift upfield. In addition, under the assumptions that only one kind of soybean ferredoxin molecule of molecular weight 10,500 and of normal optical absorbance is present, each of the fourteen contact-

* Two resonances of Figure 4 have twice the intensities of the other ten and each is considered to be two coincident resonances of unit intensity.

shifted resonances corresponds in intensity to only one-half a proton. The simplest interpretation is that instead of a single ferredoxin species, there are actually two different ferredoxins of approximately equal concentration present in the preparation.

PMR would appear to offer a particularly sensitive approach to physical differentiation between ferredoxins of different species and even to detection of multiple ferredoxins of a given species. It is not known what structural differences exist between the two ferredoxins of soybean Kent, although such differences probably reside in the amino acid sequences of the polypeptide chains. Such differences conceivably could be smaller than the four points of amino acid heterogeneity detected by Benson and Yosunobu in the sequences of ferredoxin from Leucaena glauca trees[10, 11] and as suggested by Keresztes-Nagy, Perini, and Margoliash for alfalfa ferredoxin.[12, 13] Relatively minor differences in sequences could affect through conformational perturbations orientations about C_β-S bonds of cysteine residues, and these could be manifested in the PMR spectra by large differential contact interaction shifts. These spectra, thus, may be useful for studying genetic controls of plant ferredoxins and for elucidating phylogenetic relationships of plants.

Studies are in progress on a number of other plant ferredoxins, including a variety of soybean ferredoxins.

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6. We are indebted to Dr. W. H. Mitchell of the University of Delaware for supplying us with alfalfa (Medicago sativa) for the ferredoxin preparation.

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13. We, incidentally, have not detected by PMR a heterogeneity of alfalfa ferredoxins in our preparation, but have observed different contact-shift spectra for reduced parsley ferredoxin prepared independently by A. San Pietro and by J. A. Fee and G. Palmer.