PMR and Magnetic Susceptibility Studies on *Clostridium acidi-urici* Ferredoxin

by

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SUMMARY

PMR and magnetic susceptibility studies of *C. acidi-urici* ferredoxin are reported. The magnitude and temperature dependence of the magnetic susceptibility of oxidized ferredoxin indicate extensive antiferromagnetic exchange coupling between component iron atoms. Contact-shifted resonances attributed to the β-CH_2 protons of the eight cysteine residues are observed in both redox forms of the protein. A comparison between *C. pasteurianum* and *C. acidi-urici* ferredoxins suggests a striking similarity among the magnetic, electronic, and geometrical properties of the iron-sulfur centers of the two ferredoxins.

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In a previous communication we reported results of proton magnetic resonance (PMR) and magnetic susceptibility studies on the oxidized form of the eight-iron ferredoxin from Clostridium pasteurianum.[1] These studies established that the oxidized form of the ferredoxin was paramagnetic and strongly suggested that the component iron atoms were coupled by an antiferromagnetic exchange interaction.

The ferredoxin from Clostridium acidi-urici, like that of C. pasteurianum, possesses a molecular weight of 6,000[2] and contains eight iron atoms and eight inorganic sulfur atoms.[3] The eight cysteine residues of this ferredoxin are located at the same positions along the polypeptide chain as the eight of C. pasteurianum ferredoxin.[4] Both these ferredoxins undergo a two-electron reduction with an E_o' of about -0.390 volts. We compare here the magnetic susceptibilities and contact-shift spectra of the oxidized forms of the two clostridial ferredoxins, and report the first contact-shift spectrum of a reduced eight-iron ferredoxin, that of C. acidi-urici.

METHODS AND MATERIALS

C. acidi-urici was grown and its ferredoxin was isolated as described previously.[3,5] A solution of the crystallized ferredoxin in 0.1 M $(Tris)_2SO_4$ pH 7.4 was dialyzed for 150 minutes twice against a solution of 0.1 M $(Tris)_2SO_4$ pD 7.8 in D_2O at 4°, and then concentrated sixfold by passing a stream of dry nitrogen over the resulting solution. A few crystals of DSS (sodium 2,2-dimethyl-2-silapentanesulfonate) and $(CH_3)_4NCl$ were added to the concentrated, deuterium-exchanged solution, which was immediately used for the susceptibility determination. At the conclusion of the susceptibility measurements, the protein solution exhibited an $A_{390}/A_{280} = 0.775$. Spectra were obtained on a Varian 220 MHz PMR spectrometer and internally referenced to DSS.

RESULTS AND DISCUSSION

Magnetic Susceptibility

The temperature dependence of the paramagnetic component of the solution molar magnetic susceptibility (χ_m^p) of the oxidized form of the ferredoxin from C. acidi-urici is presented in Figure 1. The susceptibility was determined by an NMR method.(6) The narrow temperature range was dictated by the extreme sensitivity of the magnetic susceptibility determination to small amounts of high-spin iron that can arise from thermal decomposition.

From Figure 1, the eight-iron ferredoxin from C. acidi-urici clearly possesses a paramagnetic component and its magnetic susceptibility over the narrow temperature range examined does not exhibit a 1/T Curie law dependence. Qualitatively, the magnetic behavior of the oxidized form of the ferredoxin from C. acidi-urici is very similar to that observed earlier for the oxidized ferredoxin from C. pasteurianum.(1) The temperature dependence of the square of the effective magnetic moment per iron atom for oxidized ferredoxin from C. acidi-urici, derived

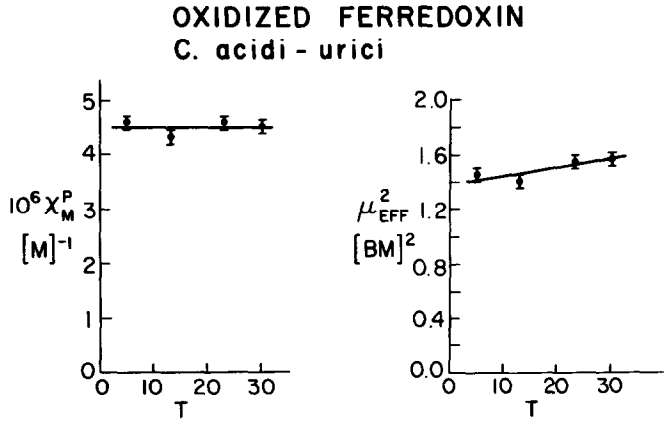

Figure 1

Temperature dependence of the paramagnetic contribution to the magnetic susceptibility (left) and the squared effective magnetic moment per iron atom (right) for oxidized C. acidi-urici ferredoxin.

from the expression

$$\mu_{eff}^2 = \frac{3kTX_m^p}{8N\beta^2} \qquad (1)$$

under the assumption that all eight iron atoms contribute equivalently to the paramagnetism, is presented on the right side of Figure 1. μ_{eff} per iron atom for the oxidized ferredoxin of C. acidi-urici varies between 1.20 and 1.26 Bohr magnetons over the accessible temperature range, values that are substantially less than even the 1.73 Bohr magnetons to be expected for only a single unpaired electron per iron atom. μ_{eff} per iron atom of oxidized ferredoxin from C. pasteurianum varied between 1.02 and 1.08 Bohr magnetons over the same temperature range.[1] This difference between the susceptibilities of the two clostridial ferredoxins may be real, but could be caused by small amounts of adventitious iron in the C. acidi-urici ferredoxin. In any event, the small but real paramagnetic susceptibilities of the oxidized ferredoxins from C. acidi-urici and C. pasteurianum, along with their temperature dependences, suggest antiferromagnetic exchange coupling among their component iron atoms.

PMR Contact Shifts

The PMR spectrum of oxidized C. acidi-urici ferredoxin in D_2O at 30°C. is presented in Figure 2. In addition to the strong, relatively narrow resonances observed in the 0 to +8 ppm region of resonance absorption, the oxidized ferredoxin exhibits a number of weak, comparatively broad resonances in the +8 to +17 ppm region that are displayed in the computer-averaged inset at the upper left of Figure 2. The resonances in the 0 to +8 ppm region are attributed to carbon-bound protons residing on side chains of amino acid residues not directly bonded to the iron-sulfur centers, and will not be further considered here.

Temperature dependences of resonances in the 1400 Hz (6.4 ppm) to 3700 Hz (16.8 ppm) region of oxidized C. acidi-urici ferredoxin are presented on the left side of Figure 3. Intensities

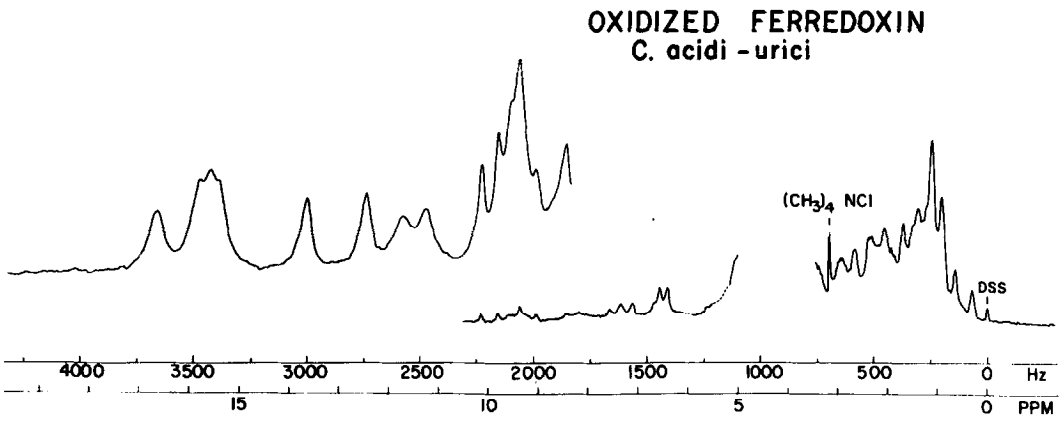

Figure 2

PMR spectrum of oxidized C. acidi-urici ferredoxin at 30°. The lower trace is a single pass spectrum with the intense HDO and Tris resonances at 1050 and 820 Hz, respectively, omitted. The upper-left insert is a 40-pass computer averaged spectrum. The ferredoxin is 8.05 mM (for $\epsilon 390 = 30{,}000\ M^{-1}\ cm^{-1}$) with $A_{390}/A_{280} = 0.775$.

in terms of protons per ferredoxin molecule are indicated to the right of each line. On the bases of their breadths, extreme low-field positions and temperature dependences, the eight resonances of unit intensity between 2,400 Hz and 3,700 Hz clearly appear to originate from a contact interaction, probably of the isotropic hyperfine variety. Eight of the nine resonances of the 1,700 Hz to 2,200 Hz range appear also to be subject to contact interaction; one of these resonances at about 2,100 Hz appears to arise from an exchangeable proton whose replacement by deuterium is very slow under the solution conditions employed.

There thus appear to be contact-shifted resonances corresponding to sixteen protons in the PMR spectrum of oxidized ferredoxin of C. acidi-urici, similar to the situation encountered for the oxidized ferredoxin from C. pasteurianum.[1] For each of these proteins the sixteen contact-shifted resonances are tentatively associated with the β-CH_2 protons of the eight component cysteine

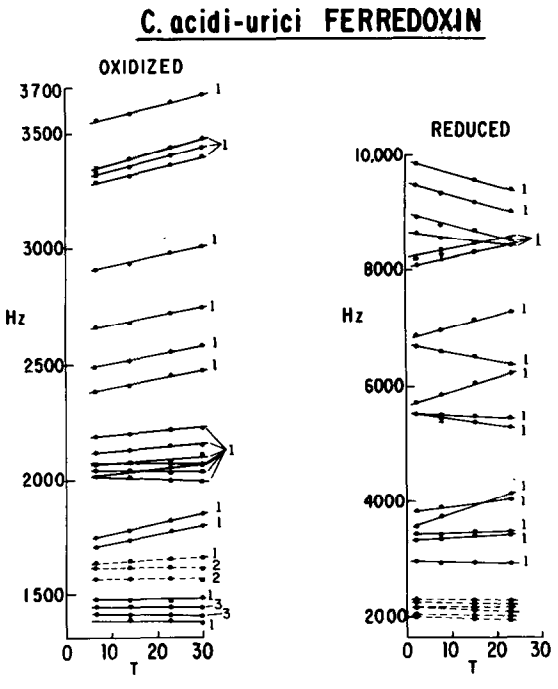

Figure 3

Temperature dependences of the low-field resonances of oxidized and reduced C. acidi-urici ferredoxin. Numbers of protons per ferredoxin molecule giving rise to certain of the resonances are indicated on the Figure. Dashed lines arise from protons slowly exchangeable against D_2O, probably buried amide NH protons.

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residues that are believed to be involved in binding the iron-sulfur moieties to the polypeptide chain. In this connection it is noteworthy that the positions of the eight cysteine residues are identical in the polypeptide chains of the ferredoxins from C. pasteurianum,[7] C. acidi-urici,[4] and C. butyricum.[8]

In our previous PMR study of the ferredoxin from C. pasteurianum, the spectrum of the reduced form was not reported because of decompositional difficulties encountered in the less stable reduced form. These difficulties subsequently were overcome, and studies on the reduced ferredoxin from C. pasteurianum will be published shortly. Here, however, we report briefly results

of studies on the reduced form of the ferredoxin from C. acidi-urici.

Temperature dependences of resonances in the 2,000 Hz to 10,000 Hz region of resonance absorption of reduced C. acidi-urici ferredoxin are shown on the right side of Figure 3. Sixteen resonances in the 2,700 Hz to 10,000 Hz region, each of an intensity corresponding to one proton, appear clearly from their temperature dependences and extreme low-field positions to owe their origin to a contact interaction. Several features appear worthy of note. Contact-shifted resonances of the reduced form range very much further to low-field than those of the oxidized form. In addition, instead of uniformly increasing with temperature as is the case for the contact-shifted resonances of the oxidized form, eight of sixteen contact-shifted resonances of reduced C. acidi-urici ferredoxin actually decrease in magnitude (move towards higher resonance fields) with increasing temperature. If assignment of the sixteen contact-shifted resonances of reduced C. acidi-urici ferredoxin to the sixteen β-CH_2 protons of the eight cysteine residues is correct, there appear, then, based on the temperature dependences of Figure 3, to be two classes of magnetic environments experienced by the side chains of the cysteine residues. The temperature dependence of contact-shifts exhibited by the β-CH_2 protons of each cysteine residue presumably reflects the local electronic and magnetic properties of the iron atom or atoms to which it is bound. A tentative conclusion, then, is that the two added electrons of reduced C. acidi-urici ferredoxin are not shared equally by the eight component iron atoms. The PMR characteristics of reduced C. acidi-urici ferredoxin are reminiscent of those encountered in the reduced forms of the two-iron plant ferredoxins[9] and in the oxidized form of the four-iron high potential iron protein from Chromatium;[10] observed contact-shifted resonances in the formally paramagnetic forms of these proteins were distributed into two classes, one of which exhibited

a positive temperature dependence, and the other a negative temperature dependence. These observations, similarly, were attributed to a nonuniform distribution of unpaired spin over the component iron atoms. The interpretation of these results is further complicated by the observations that two overlapped electron spin resonance signals are elicited from each of the C. acidi-urici[11] and C. pasteurianum[12] ferredoxins during reductive titrations. Thus, it is possible that protons associated with two separated paramagnetic centers may give rise to two classes of contact shifted resonances, differentiated by their temperature dependences. Alternatively, each center may contain the two classes of protons.

PMR studies of the two ferredoxins during reductive titrations yielded "averaged" spectra whose characteristics were intermediate between those of the fully oxidized and fully reduced proteins. Intermolecular electron exchange, perhaps complicated by intramolecular exchange, thus, was sufficiently rapid to prevent determination by NMR, at least under the solution conditions employed, of the existence of single or multiple iron sites in these proteins.

PMR and magnetic susceptibility studies thus concur in assigning an extensive antiferromagnetic spin coupling between the iron atoms of C. acidi-urici ferredoxin. Similarities between the magnetic susceptibilities and the PMR spectra of the oxidized forms of C. pasteurianum and C. acidi-urici ferredoxins are sufficiently extensive to suggest closely related structures of their redox centers. Temperature dependences of contact shifts of the reduced form of C. acidi-urici ferredoxin indicate that spin density is not uniformly distributed over all eight iron atoms. Related PMR characteristics have been observed for the reduced form of C. pasteurianum ferredoxin. No detailed structure of the iron-sulfur moiety of the eight-iron ferredoxins can be derived solely from the magnetic susceptibility and PMR results, but these results should be of value in conjunction with x-ray,

EPR, Mössbauer, and solid state magnetic susceptibility studies in progress[11] in arriving at such an end.

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