EXCHANGE COUPLING IN SPINACH FERREDOXIN DETERMINED BY RESONANCE RAMAN SPECTROSCOPY

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SUMMARY. Resonance Raman spectra have been recorded for spinach ferredoxin at 300°K and 190°K. Bands caused by antiferromagnetic exchange coupling between the irons are seen, confirming the general features of that model for the binuclear iron-sulfur clusters. Values of J_{red} of -74 cm^{-1} and J_{ox} of -172 ± 16 cm^{-1} are found. Bands associated with iron-sulfur stretching frequencies at 284, 330, and 395 cm^{-1} are also seen, and C-H stretching bands at 2840 and 2950 cm^{-1} have also been noted.

INTRODUCTION. The iron-sulfur proteins are vital constituents of many fundamental biochemical processes. Their structure and related function have been under continued investigation. Many physical properties have been determined (1) and a model has been proposed (2-4) which explains these properties well.

Previous reports of resonance Raman spectroscopy applied to the iron-sulfur (5-9) have been confined to the Fe-S vibrations. Here we report the resonance Raman spectrum of spinach ferredoxin and identify for the first time resonance Raman scattering onto the ladder of states predicted by the Gibson model for antiferromagnetically coupled iron ions. We discuss the results in relation to other physical measurements.

RESULTS AND DISCUSSION. Figure 1 shows part of the Raman spectra of oxidized and reduced spinach ferredoxin obtained with 488.0 nm excitation. The oxidized form shows bands at 284 and 395 cm^{-1} which can be correlated to those reported for adrenodoxin (7) at 297 and 397 cm^{-1}. These bands have been assigned to

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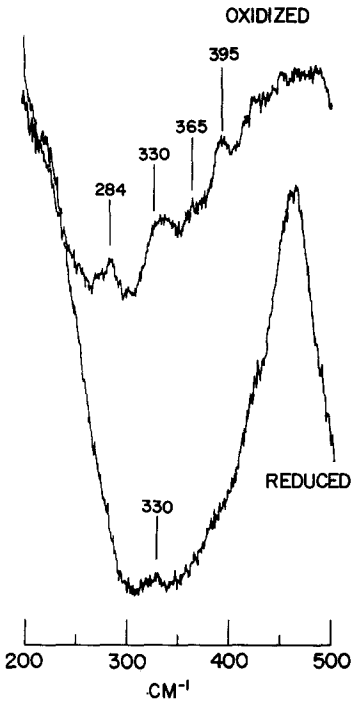

Figure 1. Resonance Raman spectra at 300°K in oxidized and reduced spinach ferredoxin excited with 488.0 nm radiation from a Kr^+ laser with Ar^+ impurity (300 mW). The spectrometer is described elsewhere (14). Excitation with the 406.7 nm Kr^+ laser (200 mW) line gives similar results but with somewhat decreased signal/noise. Sample is held in a 1 mm OD glass capillary. Sample concentration: 10 mg/ml in H_2O. Instrument conditions: scan rate 50 cm^{-1}/min; slit width 10 cm^{-1}; period 1 sec.

Fe-S (labile) stretches. A band at 330 cm^{-1} (shifted from its apparent peak at 337 cm^{-1} by the sloping baseline) can be correlated to the 350 cm^{-1} adrenodoxin band assigned to Fe-S (cysteine) stretches. In addition we detect a small signal at 365 cm^{-1}. Reduced spinach ferredoxin has only a single band at 330 cm^{-1}, with a possible band at 240 cm^{-1}. However, the large background signals near 230 cm^{-1} from water make a firm identification of this band difficult. The large signal in the 400-500 cm^{-1} region is due to the glass capillary tube which holds the sample.

In Figure 2 an expanded sweep of the Raman spectrum from 200 cm^{-1} to 3000 cm^{-1} is shown for oxidized and reduced spinach ferredoxin and for a water sample. A number of features can be seen. In the oxidized sample, bands are

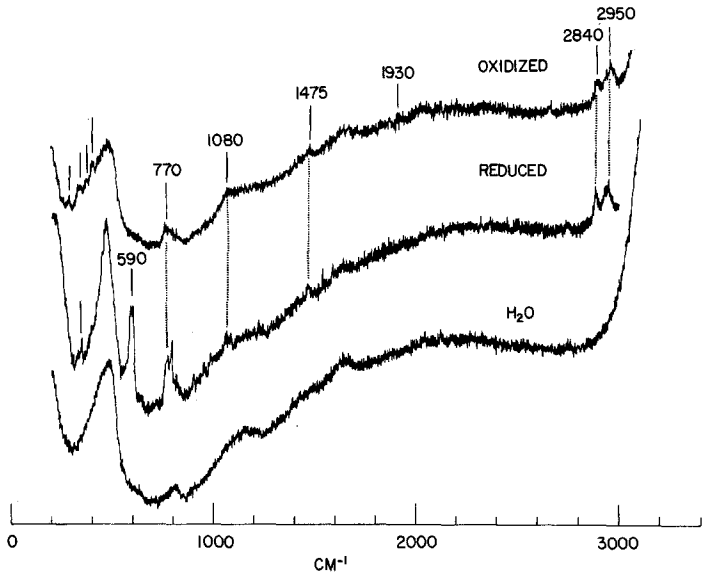

Figure 2. Extended resonance Raman spectra for oxidized and reduced spinach ferredoxin, and water at 300°K. Conditions as in Fig. 1 except scan rate 200 cm^{-1}/min; period 0.1 sec.

found below 400 cm^{-1}, as discussed above, plus additional bands at 770, 1080, 1475, and 1930 cm^{-1}. The reduced sample shows bands at 590, 770, 1070, and 1480 cm^{-1}. Other bands seen are also present in the water sample and arise from the water and from the glass capillary.

In both reduced and oxidized samples, two bands at 2840 and 2950 cm^{-1} can be seen. These are assigned to C-H stretches in the protein probably from the cysteine groups. The O-H stretch of water obscures the spectrum between about 3000 cm^{-1} and 3400 cm^{-1}. We have also scanned out to 8000 cm^{-1} but have not found any additional bands in our samples due to Fe^{+2} d-d transitions.

The Gibson model (1-4) predictions for oxidized and reduced spinach ferredoxin are given in Table I. For the oxidized ferredoxin the antiferromagnetic exchange coupling constant, J_{ox}, from magnetic suscepibility (10,11) is -182 cm^{-1}. Excitation from the ground state to the five excited states will occur at invervals of $2J_{ox}$, $6J_{ox}$, $12J_{ox}$, $20J_{ox}$, and $30J_{ox}$, as shown in Figure 3.

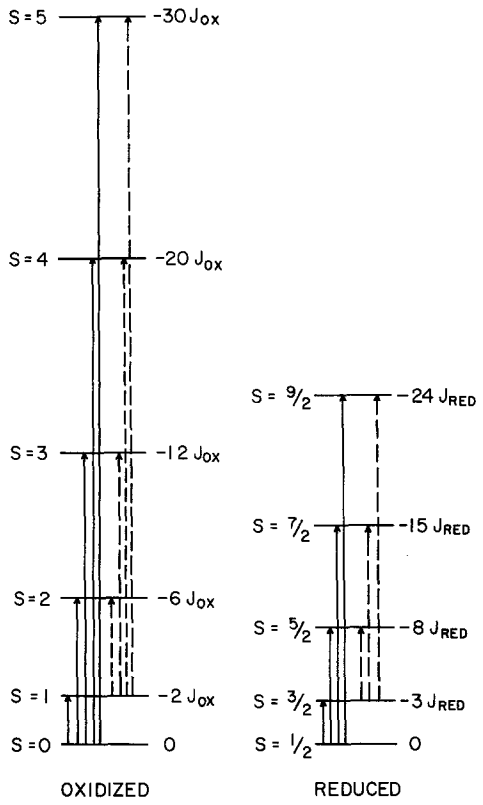

Figure 3. Ladder of states predicted for the antiferromagnetic exchange coupled irons. J_{ox} and J_{red} are the exchange coupling constants for oxidized and reduced binuclear clusters. Solid lines are transitions from the ground state. Dashed lines are transitions from the first excited state.

At room temperature, the first excited state will be thermally populated giving additional transitions at $4J_{ox}$, $10J_{ox}$, $18J_{ox}$, and $28J_{ox}$. The intensities of these transitions will diminish as the temperature is lowered and the first excited state is depopulated. Higher excited states are too far above the ground state to have appreciable population at any temperature reported here.

Similarly, reduced ferredoxin will have transitions from the ground state at $3J_{red}$, $8J_{red}$, $15J_{red}$, and $24J_{red}$. From the first excited state additional transitions at $5J_{red}$, $12J_{red}$, and $21J_{red}$ will be possible at high temperature but not at temperatures low enough to depopulate this state. The value of J_{red} lies between -80 cm^{-1} and -110 cm^{-1} from EPR (12), magnetic susceptibil-

TABLE I. Observed Resonance Raman Bands and Predictions for Antiferromagnetically Exchange Coupled Iron Atoms

Oxidized ferredoxin				Reduced ferredoxin			
Predicted (cm^{-1})[a]		Observed (cm^{-1})		Predicted (cm^{-1})[a]		Observed (cm^{-1})	
300°K	190°K	300°K	190°K	300°K	190°K	300°K	190°K
364	364	365		240	240		
728		770		400			
1092	1092	1080	1080[b]	640	640	590	590
1820		1475		960			
2184	2184	1930[c]		1200	1200		
3276				1680			
3640	3640			1920	1920		
5096							
5460	5460						

[a]Assuming $J_{ox} = -182\ cm^{-1}$, $J_{red} = -80\ cm^{-1}$.

[b]Broad.

[c]Very weak.

ity (10,11) and Mossbauer (13) measurements, with a value nearer to -80 cm^{-1} being most likely.

The experimental Raman values for oxidized spinach ferredoxin listed in Table I compare favorably with the Gibson model predictions both at 300°K and 190°K.

Figure 4 shows the Raman spectrum for oxidized spinach ferredoxin at 300°K and 190°K. A broad signal from water/glass underlies the 770 cm^{-1} band which disappears at low temperature. The band at 1080 cm^{-1} is seen to broaden and decrease in amplitude, firming up the assignments in Table I.

A simple least squares fit to the Gibson model results in $J_{ox} = 172 \pm 16$ cm^{-1} although it is to be emphasized that the uncertainty reflects the appli-

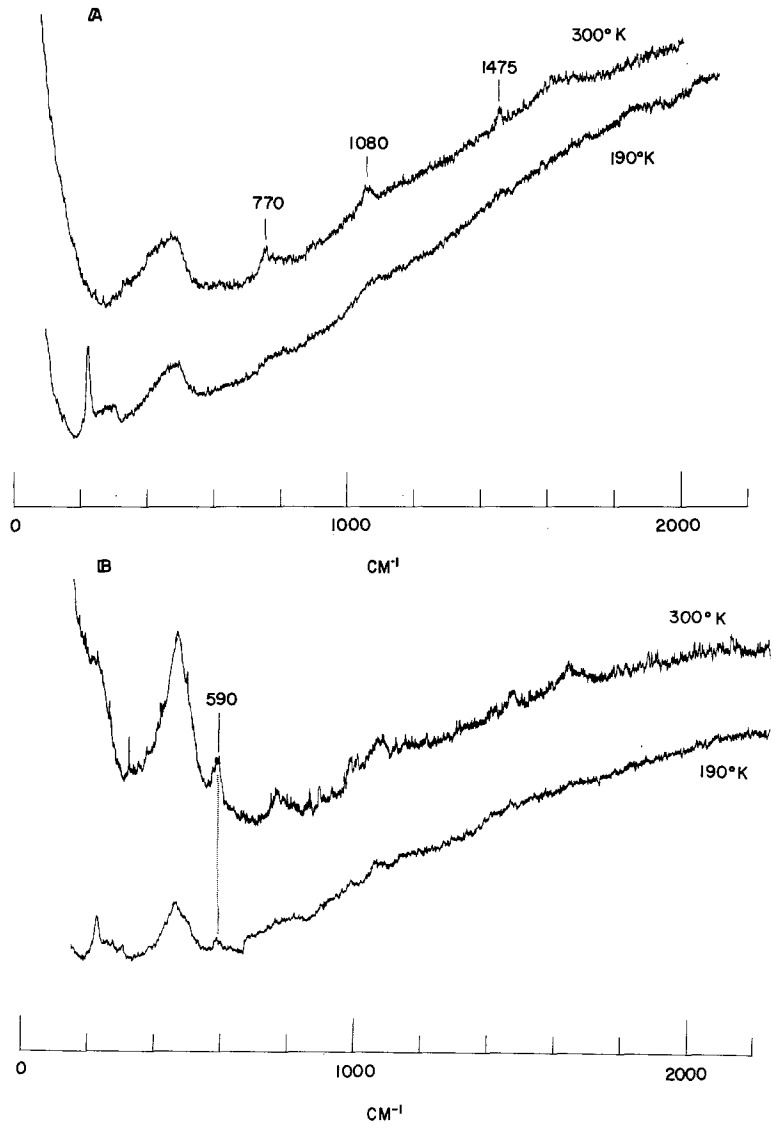

Figure 4. Temperature variation of resonance Raman spectra for (A) oxidized and (B) reduced spinach ferredoxin. Upper traces 300°K, lower traces 190°K. Lower temperatures are achieved by flowing nitrogen gas over the sample as described elsewhere (14). Temperature measurement is made with a calibrated carbon resistor placed in the cooled gas stream directly below the sample tube.

cation of the data to the model rather than uncertainties in the measured bnad positions.

In the solid dithionite reduced sample most of the observed bands are the

same as those of the oxidized form. Under our preparative conditions, the sample will be partially oxidized. These bands are therefore not taken as representative of the reduced form. This leaves only a possible band at 240 cm^{-1}, already discussed, and a band at 590 cm^{-1} which is present both at 300°K and 190°K. If we tentatively identify this band with the transition to the $8J_{red}$ level, J_{red} would have to equal -74 cm^{-1} which is close to the predicted value.

Resonance Raman spectroscopy thus supports the general idea that physical properties of the binuclear iron-sulfur proteins can be described by an antiferromagnetic exchange coupling between the irons. Our measurements are in general agreement with other techniques (10-13) which can only test the position of the first excited state. Resonance Raman spectroscopy is unique in its ability to measure the positions of higher levels in the ladder of exchange coupled states. These levels do not follow the simple model exactly. Taking the values for J which we measure at face value, it appears that the exchange coupling constants fall off with increasing temperature, which is not unexpected as thermal population of Fe-S vibrations should increase Fe-Fe distance. From the work of Ginsberg et al (15) we estimate that the decrease of J which we find could occur of the average Fe-Fe distance increased by .02 Å.

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