ANTI-FERROMAGNETIC EXCHANGE IN BEEF ADRENODOXIN AS MEASURED BY RESONANCE RAMAN SPECTROSCOPY

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1. Introduction

The two-iron ferredoxins are iron–sulfur proteins which function as one-electron redox components in a wide variety of organisms. Spinach ferredoxin functions in the electron transport system of chloroplasts. Adrenodoxin is part of the electron transport system serving in the activation of oxygen required to hydroxylate steroid molecules in mitochondria of the adrenal cortex – it transfers an electron from reduced flavoprotein to oxidized cytochrome *P*-450.

Results of chemical, magnetic susceptibility, electron spin resonance and Mossbauer studies have been used in constructing a model for the two-iron cluster. It has been proposed that each iron is tetrahedrally coordinated to four sulfur atoms – two cysteinyl sulfurs and two labile sulfide ions. Each labile sulfur bridges the two iron ions, yielding an approximately planar arrangement. The physical description of oxidized ferredoxin can be understood within an electronic scheme in which each ferric iron is high spin and in which the iron ions are antiferromagnetically (exchange) coupled to yield a total net spin of 0 in the lowest state [1]. The first excited state can be measured from the temperature dependence of magnetic susceptibility if the state is low enough to be thermally populated at temperatures below which the protein is denatured. Thus in spinach ferredoxin the temperature dependence of the magnetic susceptibility was fit to a state at 366 cm^{-1} (J = 183 cm^{-1}) for which S = 1 [2]. The second excited state (S = 2) was predicted to be at 1098 cm^{-1}, an energy three times as large. We reported earlier, resonance Raman (RR) bands corresponding to these two states in spinach ferredoxin at 365 cm^{-1} and 1080 cm^{-1} [3]. At once we demonstrated the feasibility of using the RR technique to detect these states and its ability to measure levels that are inaccessible to other physical techniques.

We report here the RR bands in adrenodoxin corresponding to the S = 1 and S = 2 states analogous to those observed in spinach ferrodoxin. In adrenodoxin, however, the energy levels of even the first excited state were unknown previously; magnetic susceptibility [4] indicated only that it was more than 700 cm^{-1} above the ground state [5]. In this protein we find RR bands at 995 cm^{-1} and 2975 cm^{-1} which indicate exchange coupling J = 497 cm^{-1}.

2. Materials and methods

Bovine adrenodoxin was prepared by the method described [6]. The absorbancy ratio, A_{414}/A_{276}, was 0.86. After lyophilization the sample was stored at −20°C until used. Spectra were taken in solutions at room temperature.

The Raman spectra were recorded on a Jobin-Yvon

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double monochromator equipped with a cooled GaAs photomultiplier and photon-counting electronics. The spectra were excited by the 413.1 nm and 476.2 nm lines of a Spectra Physics 171-01 krypton laser, and by the 488.0 nm argon impurity line in the same laser.

3. Results

The RR spectra excited at 413.1 nm, 476.2 nm and 488.0 nm between 200 cm^{-1} and 1100 cm^{-1} are displayed in fig.1. Bands at 288 cm^{-1}, 345 cm^{-1} and 390 cm^{-1} are identified as iron stretching frequencies and agree substantially with those reported [7]. In addition, we observed a band at 995 cm^{-1}. The intensity of the 995 cm^{-1} relative to the lower frequency iron–sulfur stretches is maximal in the RR spectrum excited at 488.0 nm.

The RR spectrum between 2600 cm^{-1} and 3100 cm^{-1} generated by 488.0 nm excitation is shown in fig.2. The C–H band observed in ferredoxin at 2950 cm^{-1} is centered here at 2947 cm^{-1}. The second C–H stretch observed in ferredoxin at 2840 cm^{-1} is broadened beyond recognition in adrenodoxin. In addition, however, another band is observed at 2975 cm^{-1} whose intensity is comparable to the

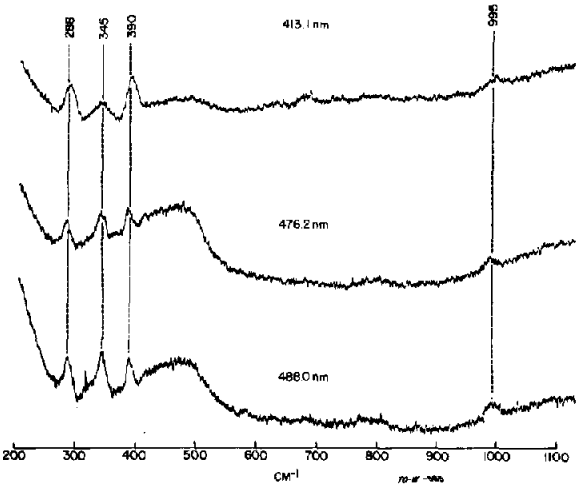

Fig.1. Resonance Raman spectra between 200 cm^{-1} and 1100 cm^{-1} of adrenodoxin excited at 488.0 nm, 476.2 nm and 413.1 nm. Power incident on the sample was about 100 mW. Instrumental resolution was 10 cm^{-1}. Scanning speed was 100 cm^{-1}; time constant was 1 s.

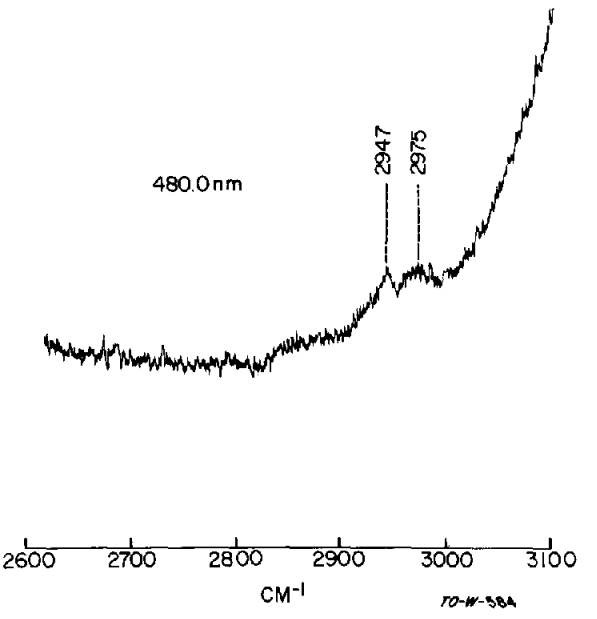

Fig.2. Resonance Raman spectrum of adrenodoxin between 2600 cm^{-1} and 3000 cm^{-1} excited at 488.0 nm. Conditions same as in fig.1 legend.

995 cm^{-1} band. Between 1100 cm^{-1} and 2600 cm^{-1} no RR bands were observed.

Both the band at 995 cm^{-1} and the band at 2975 cm^{-1} had widths of about 20 cm^{-1}; these bands were about 50% broader than the iron–sulfur stretches.

4. Discussion

In an earlier report on the RR spectra of oxidized spinach ferredoxin we observed transitions from the ground state and from the thermally-populated first excited state [3]. The frequencies of the bands fit those predicted by the Gibson model [1] surprisingly well, based on the known position of the first excited state at 366 cm^{-1} above the ground state.

In the case of adrenodoxin, it was only known that the comparable first excited state is at least 700 cm^{-1} above the ground state [5]. (Adrenodoxin remains essentially diamagnetic up to room temperature [4].) The band at 995 cm^{-1} is being assigned as the transition from the anti-ferromagnetically coupled ground

state to the $S = 1$ first excited state. The Gibson model predicts the next transition to the second excited state to be at $3 \times 995\ cm^{-1} = 2985\ cm^{-1}$. The band at 2975 cm^{-1} nearly precisely fits this frequency. (It may be of interest to note that the $\Delta S = 2$ transition in both adrenodoxin and spinach ferredoxin occurs at frequencies slightly lower than the predicted values.)

The relative intensities of the two magnetic transitions in both proteins is unusual in that the $\Delta S = 2$ band has comparable, or slightly higher intensity than the $\Delta S = 1$ band. If the $\Delta S = 2$ transition involves two spin–flip processes analogous to $\Delta S = 1$, then its intensity would normally be expected to be substantially weaker than the $\Delta S = 1$ transitions. Similar behavior has been observed in Raman scattering of magnons in anti-ferromagnets [8] and will be discussed more completely in a subsequent publication.

Preliminary data on the dependence of the RR intensity of the 995 cm^{-1} band on excitation wavelength is also displayed in fig.1. While the peak in the optical absorption spectrum of adrenodoxin at 415 nm is about 10% more intense than that at 453 nm, the intensity of the RR band at 995 cm^{-1} relative to the vibrational bands between 250 cm^{-1} and 400 cm^{-1} (or to the broad scattering of the glass capillary between 300 cm^{-1} and 550 cm^{-1}) is largest in the RR spectrum excited at 488.0 nm. The optical absorption bands in oxidized ferredoxin at 400–500 nm are best described as sulfur–iron charge transfer transitions [9]. Presumably the inequivalence of the labile sulfides and cysteinyl sulfur ligands will result in two types of charge transfer transitions. Since the exchange interaction probably involves the bridging of labile sulfide ions, the dependence of the RR intensity of the 995 cm^{-1} band on excitation wavelength suggests that the optical absorption bands are indeed labile sulfur–iron charge transfer bands, in agreement with the observation that both bands shift when these sulfur atoms are replaced with selenium [9]. Molecular orbital calculations and a more extensive excitation profile (including the 2975 cm^{-1} RR band) will be useful in verifying this supposition.

As noted above the widths of the spin–flip transitions are substantially larger than those of the iron–sulfur stretches. Extrapolation of the temperature dependence of the electron spin resonance signals of the reduced form of the protein to the oxidized form at room temperature indicates that the lifetime of the $S = 1$ state cannot be much shorter than 30 ns, which corresponds to an uncertainty-limited linewidth of less than 0.007 cm^{-1}, which is far sharper than any RR band observed in this protein. It is feasible then that the observed linewidths reflect at least in part the zero field splittings of these states. An order of magnitude estimate of the zero field splitting of the $S = 1$ state is based on the ratio ζ^2/δ where ζ is the spin-orbit coupling constant (est. 300 cm^{-1} [1]) and δ is the ligand field splitting (order 3000 cm^{-1} [10]). This puts the zero field splitting in the range of 30 cm^{-1}, close to our observed value of 20 cm^{-1}. If this analysis is correct, it would predict that the linewidths observed in our RR spectra will be temperature independent.

In summary we have demonstrated that it is possible to measure the levels of exchange-coupled states in spinach ferredoxin and adrenodoxin. Particularly in the case of adrenodoxin, this is the first measurement of any of these levels, thus providing a J value of about 497 cm^{-1}.

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