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FERREDOXIN CIRCULAR DICHROISM AT 3600 cm^{-1}

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SUMMARY

A very broad circular dichroism band is observed in reduced spinach ferredoxin at 3600 cm^{-1}. The maximum $\Delta\varepsilon$ is $+1.1\ M^{-1} \cdot cm^{-1}$. The rotational strength is $+1.0 \cdot 10^{-39}$ erg · cm^3 (+0.11 Debye–Bohr magneton); the minimum magnetic transition dipole moment is 0.14 Bohr magneton. These values are roughly consistent with expectations for a d → d transition of tetrahedrally coordinated Fe^{2+}.

No CD is detected in the oxidized protein between 5000 and 2800 cm^{-1}.

INTRODUCTION

The presence of a d → d transition near 4000 cm^{-1} in reduced spinach ferredoxin was first predicted from ESR data by Gibson and coworkers [1]. Eaton and his associates [2] subsequently demonstrated the presence in this protein of a broad but weak electronic absorption band ($f = 10^{-3}$) commencing at 5000 cm^{-1} and having a peak absorbance of approx. 200 $M^{-1} \cdot cm^{-1}$ slightly below 4000 cm^{-1}. Evidence for an absorption band of similar energy and intensity was detected in adrenodoxin and rubredoxin. Moreover, relatively intense circular dichroism (CD) was detected at the edge of these absorption bands in adrenodoxin and rubredoxin, but experimental difficulties apparently prevented CD measurements at energies less than 5500 cm^{-1} in ferredoxin. In the present paper we demonstrate a strong CD band at 3600 cm^{-1} in reduced spinach ferredoxin.

METHODS

Ferredoxin from spinach chloroplast was prepared as previously described by Petering and Palmer [3]. The absorbance ratio $A_{420\ nm}/A_{275\ nm}$, which is a measure of the structural integrity of the material, was 0.48, in good accord with literature standard [3]. Samples were dissolved in ^2H_2O rather than H_2O to minimize solvent absorbance; H_2O was removed from the samples by two cycles of freeze-drying and soaking in ^2H_2O. After the 5-h CD experiment at room temperature, the oxidized ferredoxin exhibited an optical ratio $A_{420\ nm}/A_{275\ nm}$ of 0.43. Reduction was per-

formed with excess solid $Na_2S_2O_4$. The final buffer composition was 0.15 M Tris, 1 M NaCl, pH 7.5. The oxidized ferredoxin had a concentration of 6.6 mM; the reduced sample had a protein concentration of 32 mM, based on $\varepsilon_{420\ nm} = 9400\ M^{-1} \cdot cm^{-1}$ in the oxidized material [4]. Some preliminary experiments were carried out with spinach ferredoxin from Sigma Chemical Company. The observed $A_{420\ nm}/A_{275\ nm}$ of this material was 0.42–0.45.

For infrared CD measurement the sample was placed in a sealed cell with Irtran 2 windows and a Mylar spacer. The instrument used to measure infrared CD has been previously described [5]. It employs a filter-grating monochromator (Perkin–Elmer E-1), a Perkin–Elmer wire-grid linear polarizer, and a germanium photoelastic modulator of our own design. For the studies reported here, liquid nitrogen-cooled InSb and mercury–cadmium–telluride detectors were used. In this instrument the baseline can be obtained in the absence of sample or by placing the sample between linear polarizer and the modulator (ABL position, ref. 5). Similar baselines were obtained by the two methods. The spectral bandpass (full width at half-height) was 22 cm^{-1} at 4000 cm^{-1}; this is more than adequate for the present experiment. The accuracy of the CD values obtained is estimated as +30 % due to uncertainty in absolute calibration of the electronics. The relative values of the CD at various wavelengths are substantially more accurate, however.

RESULTS

In Fig. 1 is shown the CD measured for the reduced protein. A broad, featureless band with $\Delta\varepsilon_{max} = 1.1\ M^{-1} \cdot cm^{-1}$ is found centered at 3600 cm^{-1}. The rotational strength is approx. $+1.0 \cdot 10^{-39}$ erg · cm^3 or +0.11 Debye–Bohr magneton★. CD measurements on the oxidized protein, carried out at the same pathlength but with a protein concentration one-fifth that in Fig. 1, revealed no CD between 5000 and

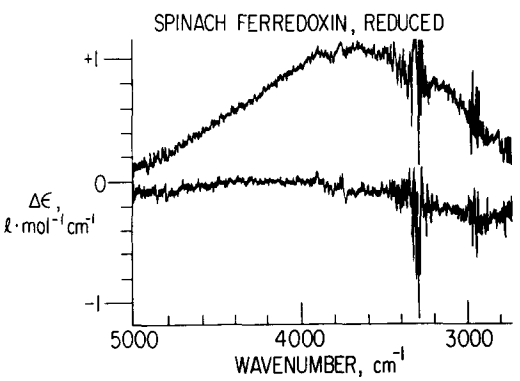

Fig. 1. The infrared circular dichroism of reduced spinach ferredoxin. The upper curve is the CD; the lower curve is the instrument baseline (ABL position of ref. 5). The large increases in noise at 3300 and 3000 cm^{-1} are due to solvent absorption. Path length 0.0087 cm; ferredoxin concentration 32 mM; temperature 25 °C.

★ We use the relation $R = 2.3 \cdot 10^{-39} \int \Delta\varepsilon d\nu/\nu$ erg · cm^3, as given by A. Moscowitz in Optical Rotatory Dispersion (C. Djerassi, ed.), McGraw-Hill, New York, 1960, Chapter 12.

2800 cm^{-1}. Further support for the absence of significant CD in the oxidized form at these spectral energies was obtained in preliminary studies with the Sigma ferredoxin. In these studies, CD was measured on a sample which was sequentially oxidized, reduced, and reoxidized while remaining in the same cell. CD was detectable between 5000 and 3800 cm^{-1} only in the spectrum of the reduced material*.

The CD data shown in Fig. 1 demonstrate unequivocally the presence of an electronic transition in reduced ferredoxin at 3600 cm^{-1}. If we use the absorption data of Eaton et al. [2] between 5000 and 4000 cm^{-1} and assume the absorption and CD bandshapes are identical, the absorption dipole strength of the 3600 cm^{-1} transition is about $6 \cdot 10^{-37}$ $esu^2 \cdot cm^2$ or 0.8 debyes. The oscillator strength is $1.2 \cdot 10^{-3}$, in accord with Eaton's result. The anisotropy factor is 0.005; the minimum magnetic transition dipole moment is 0.14 Bohr magneton. These values are suggestive of a transition which can be crudely described as magnetic dipole allowed.

CD measurements of the reduced protein were also carried out between 1000 and 750 cm^{-1} in the hope of detecting the tail of the 500 cm^{-1} $d \rightarrow d$ transition postulated from Mössbauer studies [6]. No CD was observed, but instrumental sensitivity with this sample in this spectral region was at least 10 times poorer than at 4000 cm^{-1}.

DISCUSSION

A detailed structural interpretation of these data will not be attempted here. Chemical and spectroscopic studies, which have been thoroughly reviewed [7, 8], support a structural model for ferredoxin in which each iron atom is approximately tetrahedrally coordinated to two cysteinyl and two "acid-labile" sulfur atoms. Infrared spectra of this protein reveal electronic absorption bands at 11 000, 5800 and 3800 cm^{-1} in the reduced protein, and at 10 700 cm^{-1} in the oxidized species [2]. Relatively intense CD, which supports their assignment to "$d \rightarrow d$" transitions, has now been reported for all these bands. The low-temperature optical absorption study by Slack et al. [9] of substitutional Fe^{2+} in cubic ZnS shows that, in the absence of spin-orbit effects, a $^5E \rightarrow {}^5T_2$ band near 3400 cm^{-1} ($Dq = -340$ cm^{-1}) is expected for Fe^{2+} bonded tetrahedrally to four sulfur ions. In ferredoxin, of course, the site is not precisely tetrahedral, since there are two classes of ligand as well as an adjacent Fe^{3+}; distortions from tetrahedral site symmetry are demonstrated both by Mössbauer [6] and by electron-nuclear double-resonance spectroscopy [10]. A detailed interpretation of the interactions leading to the 3600 cm^{-1} excited electronic state of ferredoxin is thus not straightforward. Our understanding would be greatly facilitated by low-temperature CD and absorption studies of the 3600 cm^{-1} band as well as the band postulated at 500 cm^{-1}.

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* G. Palmer (private communication) informs us that he and P. J. Stephens have studied the CD of oxidized and reduced spinach ferredoxin, putaridoxin, and adrenodoxin between 33 000 and 4000 cm^{-1}. They have also observed in each optical activity arising from the band at 3600 cm^{-1}. However, instrumental limitations prevented an accurate measurement of the band maximum.

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