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Creation and ESR Identification, in Single Crystals, of Synthetic Analogs of the $S=\frac{1}{2}$ States of the Fe_4S_4 Cores of the Reduced Ferredoxins and Oxidized High-Potential Proteins

J. Gloux,[a] P. Gloux,[b] B. Lamotte, and G. Rius[a]
Département de Recherche Fondamentale, Service de Physique, Centre d'Etudes Nucléaires de Grenoble, F-38041 Grenoble Cedex, France

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The synthetic equivalents of the paramagnetic states of the active sites of the ferredoxin and high-potential Fe_4S_4 proteins have been created in single crystals. This has been accomplished by induction of *in situ* redox reactions by ionizing irradiation in the crystal of the compound chosen to be initially in the diamagnetic state. This opens the way for detailed studies with ESR of the magnetic and electronic properties of these important polymetallic iron-sulfur cores.

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Many fundamental biological processes such as nitrogen fixation, photosynthesis, etc., involve nonheme iron-sulfur proteins which act as electron carriers. Their active sites are made of polynuclear iron-sulfur clusters where each iron atom is tetrahedrally coordinated to four sulfur atoms. Clusters having from one to four iron atoms have been identified.[1,2]

Model compounds have been synthesized whose structural, electronic, magnetic, and redox properties aim to approach closely those of the proteins. So, the compounds having the general formula $[Fe_4S_4(SR)_4]^{n-}$ (with R = alkyl or aryl and with $n=1$–3) are considered to be good models for the different redox states of the active sites of proteins with four-iron–four-sulfur "cubanes," such as the ferredoxins (Fd) and the high-potential (HP) iron-sulfur proteins.

Between these synthetic models and the corresponding proteins, the following one-electron-transfer correspondence has been established[3]:

$$[Fe_4S_4(SR)_4]^{3-} \rightleftarrows [Fe_4S_4(SR)_4]^{2-} \rightleftarrows [Fe_4S_4(SR)_4]^{-},$$

$$\mathrm{Fd_{red}} \rightleftarrows \mathrm{Fd_{ox}} \rightleftarrows (\mathrm{Fd_{super\,ox}}),$$

$$(\mathrm{HP_{super\,red}}) \rightleftarrows \mathrm{HP_{red}} \rightleftarrows \mathrm{HP_{ox}}.$$

(Note that the states of the Fd and HP proteins written in parentheses are only observed under *in vitro* conditions.)

For the iron-sulfur core itself, we have then

$$[Fe_4S_4]^{+} \rightleftarrows [Fe_4S_4]^{2+} \rightleftarrows [Fe_4S_4]^{3+}.$$

The $[Fe_4S_4]^{2+}$ state is diamagnetic at low temperature. The $[Fe_4S_4]^{+}$ and $[Fe_4S_4]^{3+}$ states are paramagnetic with, almost always, $S=\frac{1}{2}$ fundamental spin states. They are characterized, respectively, by an isotropic part of the $\tilde{g}$ tensor (g_{av}) which is lower and greater than 2.

The $[Fe_4S_4]^{+}$ and $[Fe_4S_4]^{2+}$ clusters have been extensively studied in proteins as well as in synthetic models. By contrast, since it has not been possible up to now to synthesize stable $[Fe_4S_4(SR)_4]^{-}$ compounds, the information available concerning the $[Fe_4S_4]^{3+}$ state comes only from the very few studies of HP_{ox} proteins.[4]

Among physical methods, electron-spin resonance (ESR) is certainly one of the best used to identify and study these paramagnetic states, mainly through the principal values of their $\tilde{g}$ tensor. But the full potential of ESR spectroscopy has not been attained because either proteins or model compounds have only been studied in nonoriented systems (i.e., in frozen solutions or as polycrystalline powders). Under these conditions, no directional information has been gained which could be related to the geometrical structure of the cluster, which is generally known from x-ray studies.

We want to report that we have succeeded, to our knowledge for the first time, in creating in *single crystals* the *two* paramagnetic states $[Fe_4S_4]^{+}$ and $[Fe_4S_4]^{3+}$ of the cluster. For this purpose we have induced in the single crystals of the dianion model compound, by using irradiation with gamma rays, the following *in situ* reactions:

$$[Fe_4S_4]^{2+} \rightarrow [Fe_4S_4]^{3+} + e^{-},$$

$$e^{-} + [Fe_4S_4]^{2+} \rightarrow [Fe_4S_4]^{+}.$$

Since the clusters are able to undergo easy electron transfers it was indeed likely that they would be good traps for the oxidized and reduced species created by ionizing radiation in the crystal bulk. These paramagnetic species are then created under the best conditions for detailed ESR studies since they are diluted at low concentration in the crystal which is diamagnetic at low temperature.

We have chosen to study the compound $[Fe_4S_4(SC_6H_5)_4](NBu_4)_2$. It was prepared following the procedure initiated by Averill *et al.*[5] and subsequently improved by Christou and Garner.[6] The compound crystallizes at room temperature in the monoclinic system, space group $P2_1/n$ with $Z=4$.[7] It exhibits a phase

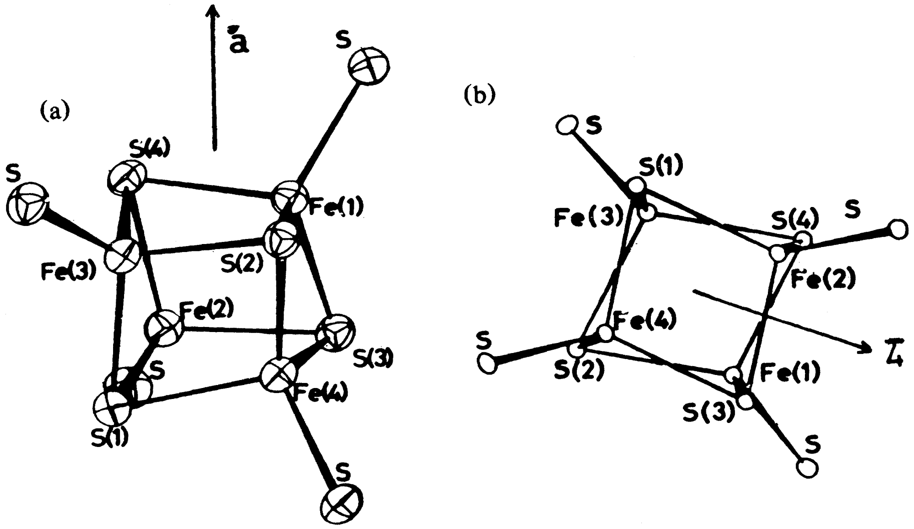

FIG. 1. (a) Geometry of the iron-sulfur core in $[Fe_4S_4(SC_6H_5)_4](NBu_4)_2$ deduced from the x-ray structure at room temperature (Ref. 7). (b) The corresponding cubane geometry as viewed in the *a* projection.

transition around 240 K which is rather unusual, going from the monoclinic phase at room temperature towards an orthorhombic phase at low temperature.[7] We were able to obtain single crystals large enough for ESR at 9 GHz ($5 \times 3 \times 0.3$ mm^3). The crystals are plate shaped with well developed *ab* faces and they grow preferentially along the *a* axis direction.

The crystal structure[7] shows that the $[Fe_4S_4]^{2+}$ core is of the usual cubane type [see Fig. 1(a)]. It has an idealized, nonimposed, D_{2d} symmetry with four short and eight long Fe-S bonds which are approximately parallel and perpendicular, respectively, to the $\bar{4}$ axis of the distortion. The overall structure is very similar to that found in the $[Me_4N]$ salt,[8] but with a somewhat larger distortion of the four-iron tetrahedron. The *a* axis of the crystal is nearly perpendicular to the Fe_1Fe_3 and Fe_2Fe_4 directions of the cubane which are themselves mutually perpendicular.

The crystals used for this study, prior to gamma irradiation, were nearly free from paramagnetic impurities in the limit of ESR sensitivity. After irradiation at room temperature (generally 2 h with ^{60}Co source; dose, 2 mrad), the sample is quickly immersed in liquid nitrogen in order to quench the crystal in the monoclinic phase, thus avoiding the phase transition. It is then transferred to the variable-temperature system in the ESR spectrometer and studied in the temperature range 4–120 K as a function of orientation of the crystal with respect to the magnetic field.

The ESR spectra so obtained contain many anisotropic lines. They spread over about 500 G, are approximately centered around $g = 2$, and are stable at low temperature. We have not observed any low-field line characteristic of $S \geqslant \frac{3}{2}$ spin states. Two sets of relatively intense lines that we label *A* and *B* exist in all the crystals that we studied; moreover, they seem to be correlated when the irradiation dose is varied. The lines of set *A* broaden out at about 50 K; they are best detected at 15–20 K with 10 mW of microwave power. The lines of set *B* are strongly saturated at low temperature; at 120 K they are still detectable while all the other lines have broadened out. The linewidths, which are anisotropic, have a mean value of 15 G for set *A* and 10 G for set *B*.

We could obtain the complete angular variation of the two sets of lines in the three perpendicular planes *a*-*b*, *b*-c^*, and c^*-*a* (Fig. 2). Theoretical curves that fix

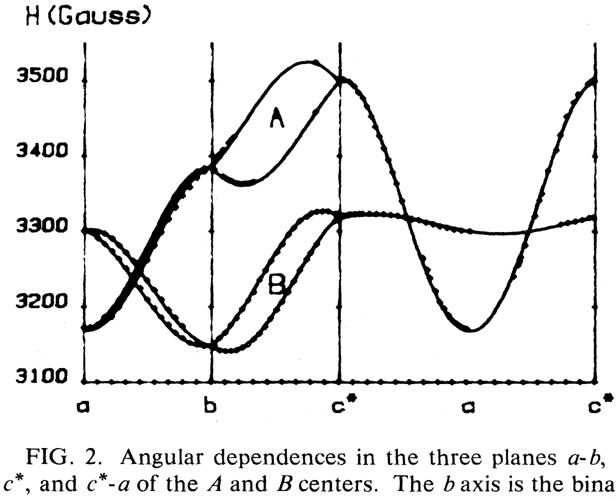

FIG. 2. Angular dependences in the three planes *a*-*b*, *b*-c^*, and c^*-*a* of the *A* and *B* centers. The *b* axis is the binary axis. The dots correspond to the experimental measurements and the continuous lines to the theoretical fits.

TABLE I. g values of the A and B centers compared with those of the corresponding paramagnetic states of the proteins.

		g_1	g_2	g_3	g_{av}	Reference
A center	$[Fe_4S_4]^+$	2.089	1.969	1.877	1.978	This work
Fd_{red}	$[Fe_4S_4]^+$	2.06	1.93	1.88	1.96	9
B center	$[Fe_4S_4]^{3+}$	2.108	2.006	1.987	2.034	This work
HP_{ox}	$[Fe_4S_4]^{3+}$	2.12	2.04	2.04	2.07	4
		2.088	2.055	2.04	2.06	

the experimental angular dependences well are calculated from a simple electronic Zeeman Hamiltonian:

$$\mathscr{H} = \mathbf{H}\cdot\tilde{\mathbf{g}}\cdot\mathbf{S}, \quad S=\tfrac{1}{2}.$$

Table I gives the principal g values that we have obtained for the A and B centers. It contains also the principal g values of a typical reduced ferrodoxin and of an oxidized HP protein.

The extent of anisotropy (g_1-g_3) of the two centers A and B, together with the fact that we never observe resolved proton hyperfine interactions for them, proves at first that they cannot be free radicals created by the ionizing radiation in the organic parts of the compound. By comparing the principal g values obtained for the A center with those of the reduced ferrodoxin, we can verify that they are similar, with, in particular, g_{av} values *lower than 2.* Thus we identify the A center with the $[Fe_4S_4]^+$ state of the iron-sulfur core in the crystal. The small differences found between the g values of the A center and of the related reduced ferrodoxin are of the same order as the dispersion in the g values that exists among different reduced ferrodoxins or synthetic models.

In the same way, we can identify the B center with the $[Fe_4S_4]^{3+}$ state of the iron-sulfur core. Just as the $[Fe_4S_4]^{3+}$ states in the HP protein of chromatium vinosum,[4] it presents a g_{av} value *greater than 2,* a quasiaxiality of the $\tilde{g}$ tensor, and a g_1 value reaching about 2.1. The g_2 and g_3 values are somewhat different from those of the protein, but it is known that these g values are solvent dependent.[10] Thus, we think that, in the present state of knowledge, our assignment of the B center to the $[Fe_4S_4]^{3+}$ state is quite reasonable.

The dependences with temperature of the linewidths and of the related electronic relaxation times of the two centers are also in good qualitative agreement with those reported for the two kinds of proteins. In effect, different authors[4,10,11] report that the $[Fe_4S_4]^{3+}$ state is observed without broadening at higher temperature than the $[Fe_4S_4]^+$ state. It is also considered that, towards lower temperatutes, its lines begin to saturate at a higher temperature than those of the $[Fe_4S_4]^+$ state. These tendencies which are obeyed even more markedly for the A and B centers thus support the attributions that we have made above.

A detailed analysis of the g-tensor directions, in connection with the geometry of the Fe_4S_4 core, is beyond the scope of this Letter and will be published later. However, we notice already that one $\mathbf{g}_1, \mathbf{g}_2, \mathbf{g}_3$ orthogonal set of axes obtained, i.e., the one relative to the tensor of the A center, fits particularly well with the geometry of the cluster core. This direction $\mathbf{g}_1$ is nearly perpendicular to the two directions Fe_1Fe_3 and Fe_2Fe_4. Since there exist two magnetically different sites in the crystal, depending on the choice of the site the $\mathbf{g}_2$ and $\mathbf{g}_3$ directions are, respectively, nearly parallel either to the Fe_2Fe_4 and Fe_1Fe_3 directions or to the commune perpendiculars to the Fe_1Fe_2 and Fe_3Fe_4 directions (i.e., the $\bar{4}$ axis of the distortion of the original cubane) and to the Fe_1Fe_4 and Fe_2Fe_3 directions [see Fig. 1(b)].

In conclusion, the ensemble of the results reported here convinces us that we are really observing the reduced and oxidized states of the cluster. This then gives the possibility of our studying by ESR, *in single crystals,* the *two* $[Fe_4S_4]^+$ and $[Fe_4S_4]^{3+}$ paramagnetic states of the cubane core. Thus it enables the measurement of their complete $\tilde{g}$ tensors and subsequently allows examination of their relation to the geometry and the bond directions of the cubane. Moreover, this illustrates how such studies of ESR in single crystals offer the possibility of disentangling and studying separately the different paramagnetic species, which would be nearly impossible to distinguish by an analysis of the corresponding complex powder spectrum.

In our opinion, this approach opens a new way to obtain detailed knowledge of the electronic and magnetic structures of these important entities.

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(a) Also at Université Scientifique et Médicale de Grenoble, Grenoble, France.

(b)Also at Centre National de la Recherche Scientifique, Paris, France.

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