SYNTHETIC ANALOGS OF THE ACTIVE SITES OF IRON-SULFUR PROTEINS. VIII. SOME ELECTRONIC PROPERTIES OF $[Fe_4S_4(SR)_4]^{3-}$, ANALOGS OF REDUCED BACTERIAL FERREDOXINS

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SUMMARY

One-electron reduction of the synthetic tetramers $[Fe_4S_4(SR)_4]^{2-}$ (R = CH_2Ph, Ph), by chemical and electrochemical methods affords the corresponding trianions. The collective results from optical, electron paramagnetic resonance, and Mössbauer spectroscopy, when compared to available data for reduced 4-Fe and 8-Fe ferredoxin proteins and super-reduced Chromatium high-potential protein, reveal that $[Fe_4S_4(SR)_4]^{3-}$ are electronic analogs of the $[Fe_4S_4(S\text{-}Cys)_4]$ active sites of these proteins.

INTRODUCTION

The synthetic tetramer dianions $[Fe_4S_4(SR)_4]^{2-}$ have been shown to be close structural and electronic representations of the active sites of oxidized 4-Fe and 8-Fe bacterial ferredoxin (Fd_{ox}) and reduced high-potential (HP_{red}) iron proteins (1-4). Although formal oxidation state considerations imply that the dianions contain 2Fe(II) and 2Fe(III), it has recently been shown by a variety of techniques that the iron sites are, in fact, indistinguishable (5). The four-membered electron transfer series below has been established by voltammetry in non-aqueous media (4). The relationships between proteins and analogs indicated in column (a) are supported by comparison of various electronic properties and the D_{2d} cubane-type structures of the representative R = CH_2Ph (2) and Ph (6) dianions, which are closely similar to those of Fd_{ox} and HP_{red} iron-sulfur clusters (7). One-electron reduction per active site of the proteins in (a) affords Fd_{red} and the recently discovered "super-reduced" form of the high potential protein, $HP_{s\text{-}red}$ (8). Available evidence indicates that the clusters in these two proteins are electronically

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$$\begin{tikzcd}
{[}Fe_4S_4(SR)_4{]}^{4-} \arrow[r, leftrightarrow] & {[}Fe_4S_4(SR)_4{]}^{3-} \arrow[r, leftrightarrow] & {[}Fe_4S_4(SR)_4{]}^{2-} \arrow[r, leftrightarrow] & {[}Fe_4S_4(SR)_4{]}^{1-} \\
& Fd_{red} \arrow[r, leftrightarrow] & Fd_{ox} & \\
& HP_{s\text{-}red} \arrow[r, leftrightarrow] & HP_{red} \arrow[r, leftrightarrow] & HP_{ox} \\
& (b) & (a) &
\end{tikzcd}$$

related. Here we report results from optical, e.p.r., and Mössbauer spectral studies which support the relationships in column (b), viz., that $HP_{s\text{-}red}$ and Fd_{red} possess the total oxidation level unambiguously defined by $[Fe_4S_4(SR)_4]^{3-}$.

MATERIALS AND METHODS

Salts of $[Fe_4S_4(SR)_4]^{2-}$ (R = CH_2Ph, Ph) were synthesized by the method described elsewhere (2). The species $[Fe_4S_4(SR)_4]^{3-}$ were not isolated but were generated in solution by reduction of dianions using three methods; all manipulations were performed in a pure nitrogen atmosphere. Employing a Princeton Applied Research Model 170 Electrochemistry System solutions of the trianions in acetonitrile were obtained by constant potential electrolysis (c.p.e.) at a platinum gauze electrode with simultaneous coulometry and monitoring of the optical spectra. The spectral cell is similar to that previously described (9). Applied potentials were ca. 0.2 V more cathodic than $E_{1/2}$ values for the 2-/3- reduction (4). Electrolyzed solutions contained 0.05 M $(n\text{-}Pr_4N)(ClO_4)$ supporting electrolyte. Solutions of the dianions in DMF were treated with an ca. 2-fold excess of a THF* solution of the acenaphthalene radical anion ($ACN^{\dot{-}}$) and the resulting solutions frozen. The half-wave potential for $ACN/ACN^{\dot{-}}$ (-1.62 V in DMF vs. saturated calomel electrode) is intermediate between 2-/3- and 3-/4- potentials of the tetramers (4) and thus $ACN^{\dot{-}}$ effects reduction to the trianion but not the tetraanion. Dianions in acetonitrile or benzonitrile were reduced by stirring for several minutes over 1% sodium amalgam; prolonged exposure to the amalgam led to further reduction and

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Abbreviations: THF, tetrahydrofuran; ACN, acenaphthalene; DMF, N,N-dimethylformamide

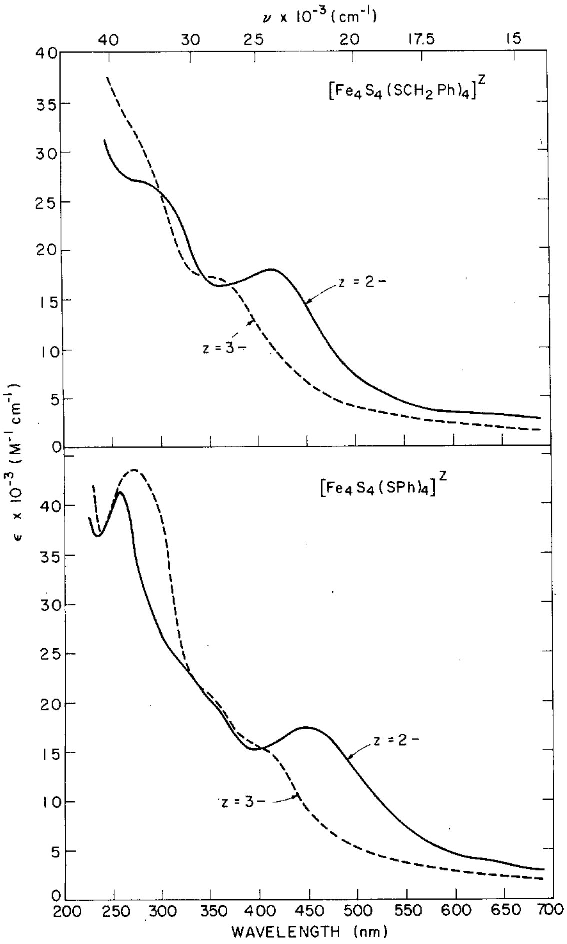

Figure 1. Optical spectra of dianions and trianions of R = CH_2Ph and Ph tetramers in acetonitrile solution. The trianions were electrochemically generated _in situ_ by c.p.e. of the dianions. The spectral changes upon electrolysis correspond to the passage of 1.0±0.05 faraday/mol tetramer.

apparent decomposition of the tetramer. Mössbauer spectra were determined on frozen solutions with a constant acceleration spectrometer operating in the normalized mode and a ^{57}Co in Rh source held at the same temperature as the absorber. Magnetic field measurements were made with a superconducting magnet in the longitudinal configuration. Isomer shifts are referenced to Fe metal. E.p.r. spectra were obtained with a Varian X-band spectrometer equipped with a Helitran liquid helium refrigeration system allowing measurements from room temperature to 5°K; samples were handled in Suprasil quartz tubes. The precision of the g-value measurements ($\Delta g = 0.002$) was limited by the width of the e.p.r. absorption line.

RESULTS AND DISCUSSION

Optical Spectra. Controlled potential electrolysis of $[Fe_4S_4(SCH_2Ph)_4]^{2-}$ and $[Fe_4S_4(SPh)_4]^{2-}$ in acetonitrile solution with simultaneous spectral monitoring demonstrates that decreases in the absorbances at 417 and 448 nm, respectively, occur concurrently with reduction. The final spectra (Fig. 1), which are associaed with coulometric n values of 1.0±0.05 and are unchanged upon exhaustive electrolysis, lack the original maxima in the visible and show increased absorbance in the ultraviolet region. Three cycles of reduction and back-oxidation incur an average loss of intensity in the 417 and 448 nm dianion peaks of 5%/cycle, indicating that the 2-/3- redox process is effectively reversible. While these peaks are somewhat red-shifted compared to Fd_{ox} spectra in aqueous solution, the spectral changes concomitant with the one-electron reduction are quite similar to those observed upon dithionite reduction of *Cl. acidi-urici* Fd_{ox} (10). In that and other cases the spectra in the visible revert to a nearly featureless form with an *ca.* 50% diminution in intensity. Reduction of the dianion in acetonitrile or benzonitrile with 1% Na/Hg gave a rapid (*ca.* 2 min) color change, most noticeable for R = Ph. Spectra of solutions separated from the amalgam at this point were the same as those obtained following reduction by c.p.e.

E.p.r. Spectra. The reduction product of $[Fe_4S_4(SCH_2Ph)_4]^{2-}$ has been the more thoroughly studied. The axial spectrum obtained by reduction with 1% Na/Hg in acetonitrile is shown in Fig. 2. Virtually identical spectra at 5-6°K have been obtained for reduced species generated by other means, as shown by the following principal g-values (±0.01): ACN*, DMF/THF, 1.92, 2.04, $g_{av} = 1.96$; c.p.e., acetonitrile and DMF, 1.93, 2.04, $g_{av} = 1.97$. These features are associated with $[Fe_4S_4(SCH_2Ph)_4]^{3-}$, whose spectrum is markedly temperature dependent. Relative peak-to-peak heights of the high field signal in Fig. 2 decrease smoothly from 1.00 at 5°K to 0.02 at 52°K and are reversible with temperature. Entirely similar behavior has been found for the trianion generated by amalgam reduction in benzo-

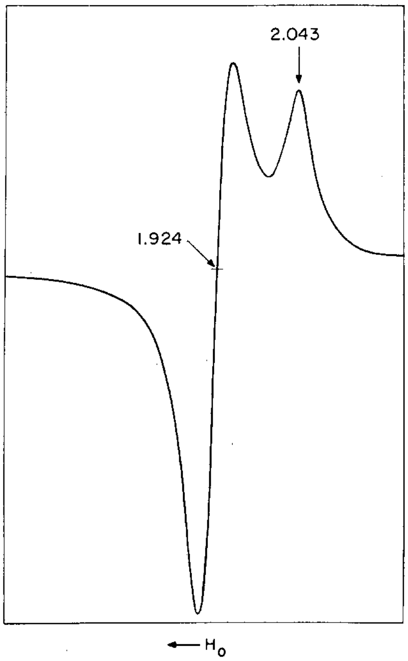

Figure 2. E.p.r. spectrum at 5°K of $[Fe_4S_4(SCH_2Ph)_4]^{2-}$ generated by reduction of a 10^{-3} M acetonitrile solution of the dianion with 1% Na/Hg. g-values are indicated and g_{av} = 1.964.

nitrile; at 5°K, g = 1.923, 2.041 and g_{av} = 1.963. E.p.r. spectra of Fd_{red} proteins are characterized by a signal at g ~1.94 and g_{av} <2. Available results on 4-Fe proteins reveal rhombic spectra (11, 12); for Bacillus polymyxa Fd_{red} g = 1.88, 1.92, 2.06 and g_{av} = 1.95 (11). However, the HP_{s-red} spectrum is axial with g = 1.93, 2.04 and g_{av} = 1.97 (8). The temperature dependence of the trianion spectrum is clearly similar to those of the proteins. For B. polymyxa Fd_{red} above 25°K signals rapidly become unobservable (11), the spectrum of HP_{s-red} is detectable only below 35°K (8), and the spectrum of Cl. pasteurianum Fd_{red} is well developed only below 77°K (13). Both axial and rhombic spectra have been found for $[Fe_4S_4(SPh)_4]^{2-}$ reduction products depending on the method of preparation. For example, treatment with $ACN^{\dot{-}}$ affords an axial spectrum (g = 1.92, 2.06) whereas amalgam reduction in benzonitrile yields a rhombic spectrum (g = 1.86, 1.94, 2.06, g_{av} = 1.95).

Mössbauer Spectra. Iron-57 spectra were determined on samples reduced with $ACN^{\dot{-}}$. Spectra of $[Fe_4S_4(SCH_2Ph)_4]^{2-,3-}$ at 77°K are shown in Fig. 3a,b. Compared to the

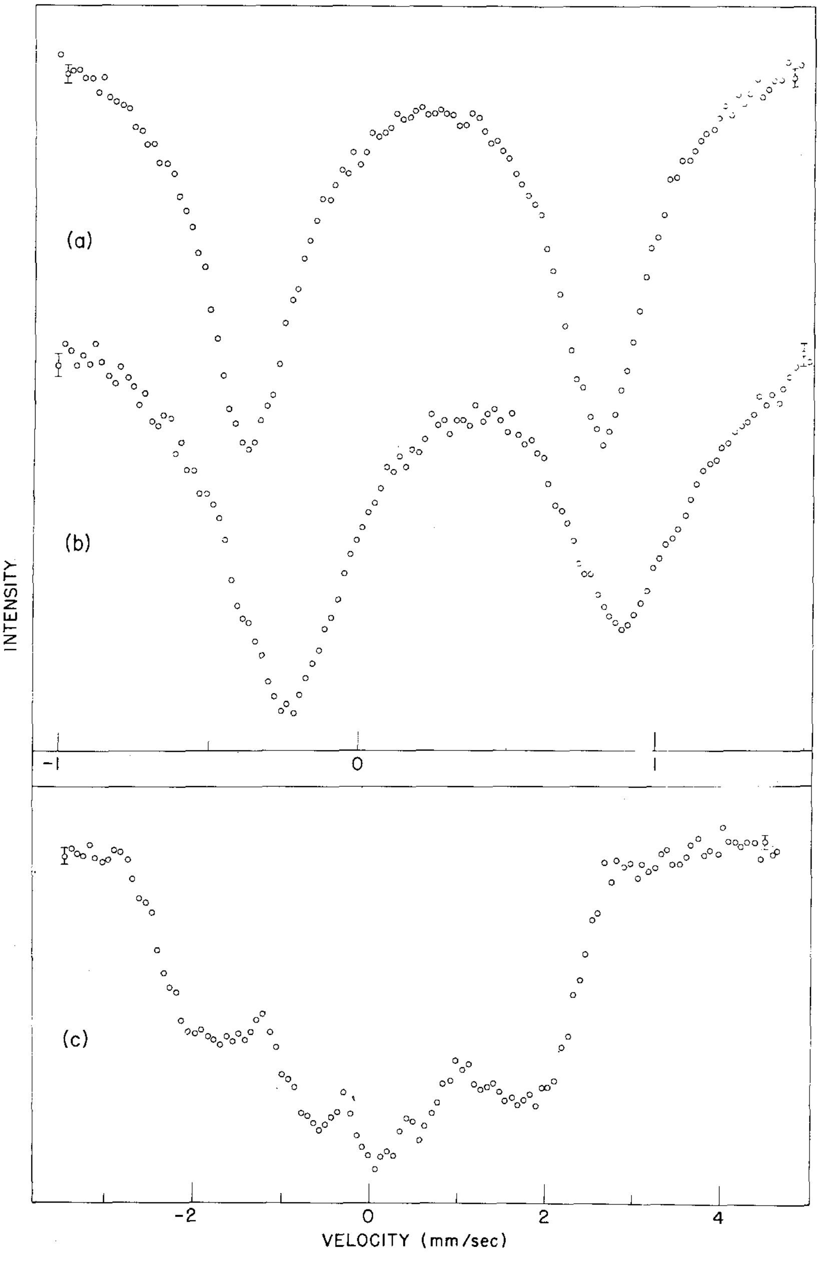

Figure 3. Mössbauer spectra of $[Fe_4S_4(SCH_2Ph)_4]^z$ at 77°K: (a) z = 2- in DMF; (b) z = 3- in DMF/THF. (c) Spectrum of $[Fe_4S_4(SCH_2Ph)_4]^{3-}$ in DMF/THF at 4.2°K with a longitudinal magnetic field of 1 kOe. Absorption lines at +0.7 and -0.4 mm/sec are due to residual dianion.

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solid, parameters of the dianion are virtually unchanged in solution (δ = 0.36 mm/sec, ΔE_Q = 1.25 mm/sec, 77°K) except for the linewidth Γ, which increased slightly to 0.32 mm/sec. For the reduced species at 77°K, δ = 0.48 mm/sec, ΔE_Q = 1.15 mm/sec, and Γ = 0.45 mm/sec indicating significant differences in isomer shift and linewidth compared to the dianion. Similar results were obtained for $[Fe_4S_4(SPh)_4]^{2-,3-}$. At 4.2°K the dianion solution spectrum consists of a single quadrupole doublet, essentially unchanged from the 77°K spectrum. Application of an external magnetic field H_0 results in a spectrum identical to that obtained with a crystalline sample in which the field at the nucleus $H_n = H_0$ (5). For the trianion at 4.2°K, the $H_0 = 0$ spectrum consists of a very broad asymmetric absorption indicative of hyperfine interaction between ^{57}Fe nuclei and a slowly relaxing electronic spin. Application of a small longitudinal field (H_0 ~1 kOe) affords a sharpening of the spectrum into hyperfine components (Fig. 3c). Application of fields H_0 up to 80 kOe results in a decrease of the overall splitting, indicative of a negative hyperfine field of the order of 100 kOe at the iron sites.

Mössbauer measurements on HP_{red} and Fd_{ox} proteins reveal an apparent quadrupole doublet (14-18) somewhat broadened compared to the dianions. Parameters for the two types of proteins are rather similar and fall in the ranges δ ~0.32-0.46 mm/sec and ΔE_Q ~1.1-1.3 mm/sec at 4.2°K (14-17). Values for R = Ph and CH_2Ph dianions are within these invervals (1,5). For Fd_{red} proteins there is less consistency among the available data. The most recent work indicates that the 77°K spectra consist of a quadrupole doublet with δ ~0.5-0.6 mm/sec and ΔE_Q ~1.1-1.4 mm/sec. At 4.2°K magnetic hyperfine interaction, due to paramagnetic relaxation, is observed (16,17). Mössbauer parameters of the trianions are comparable with the currently available values for Fd_{red}. Data for $HP_{s\text{-}red}$ have not been reported.

The near invariance of quadrupole splitting in the dianions and trianions implies that no major structural changes in the environment of the iron atoms occur upon reduction. The observed increase of isomer shift upon reduction suggests that the electron is added to an orbital of substantial d-character centered on the iron atoms. The interpenetration of the 3d and 3s, 4s orbitals is such that an increase in electron density in the former actually shields the nucleus from electrons in the latter, resulting in a net decrease in electron density at the nucleus. The trianion formally contains 3Fe(II) + Fe(III) and its isomer shift may be compared with δ = 0.54 mm/sec (195°K) found for $Ba_6Fe_8S_{15}$, which contains tetrahedral Fe-S_4 units with an average iron oxidation state of 2.25 (20). The magnetic hyperfine structure in the trianion spectrum, as well as the e.p.r. spectra, show that the electronic ground state is paramagnetic and that the electron spin relaxation time is of the order of the ^{57}Fe nuclear Larmor precession time. This result is expected because the ground state of the dianion is singlet (nonmagnetic) (1). The observed line broadening in the trianion may reflect structural or electronic differences among iron atoms in the Fe_4S_4 core. However, such differences, if any, are small. The unpaired electron is concluded to interact with all four atoms; localization on one or two metal sites is ruled out.

The optical spectral, e.p.r., and Mössbauer results establish the relationship between total oxidation levels of synthetic trianions and the protein active sites in column (b) of the electron transfer series above. The relationships shown in these series are equivalent to the three-state hypothesis of protein oxidation levels proposed by Carter _et al_. (7). Further work will involve examination of factors influencing axial _vs_. rhombic e.p.r. spectra, the question of strict equivalence of iron atoms in the trianion, and the relationship of these findings to electron distribution in the presumably less symmetric iron-sulfur clusters of the reduced proteins.

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