interactions which are not necessarily apparent from other types of physical measurement.

In support of our interpretations, in the crystal of Δ-*mer*-Co(L-ala)$_3$, two short NH···O separations (~2.6 Å) are observed for a pair of ligands, and in addition, the NH_2 and CH_3 orientations in the crystal are quite similar to those deduced from the solution VCD spectra based on competing ring current effects.[29] Although quantitative determination of solution structures is not as yet possible, it is clear that through the ring current mechanism, the VCD spectra for these complexes have provided detailed stereochemical information on the most abundant solution conformations.

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Communications to the Editor

Resonance Raman Spectra of the [2Fe-2S] Clusters of the Rieske Protein from *Thermus* and Phthalate Dioxygenase from *Pseudomonas*

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The iron-sulfur protein of the mitochondrial cytochrome bc_1 complex[1-3] and the photosynthetic cytochrome b_6f complex,[1-3] called the Rieske protein, contains a unique [2Fe-2S] cluster. Its characteristic spectral features have revealed its presence, as well, in the plasma membrane of several bacterial species[1-3] and in certain bacterial oxygenases (cf. ref 4). Recently, the protein from *Thermus thermophilus* was obtained in high purity, found to have M_r = 20000 and contain four Fe, four S^{2-}, and four cysteine residues.[4] Mössbauer and other data[4] gave strong evidence for the presence of two essentially identical [2Fe-2S] clusters, suggesting that each cluster is coordinated to two of the available cysteine residues. Within a cluster, both irons are high spin and each appears to reside in a slightly different coordination environment. An ENDOR study[5] demonstrated the presence of Fe-N bonds, probably histidine imidazoles, but gave no evidence on either the number of nitrogenous ligands or their distribution on the cluster. In this paper we describe a resonance Raman (RR) study of these novel iron-sulfur-nitrogen clusters which provides evidence for an asymmetric distribution of Cys and N ligands on the cluster. The systems examined were *Thermus* Rieske protein (TRP) and phthalate dioxygenase (PDO) from *Pseudomonas cepacia*; we compare the RR spectra of these proteins to that of spinach ferredoxin (SFD).

The [2Fe-2S] clusters of PDO and several other NADH-dependent dioxygenases appear to be structurally similar to those of TRP.[4,6] However, there are differences. For example, the midpoint potentials of the dioxygenases are normally less than -100 mV,[7,8] while the midpoint potentials of the clusters in Rieske

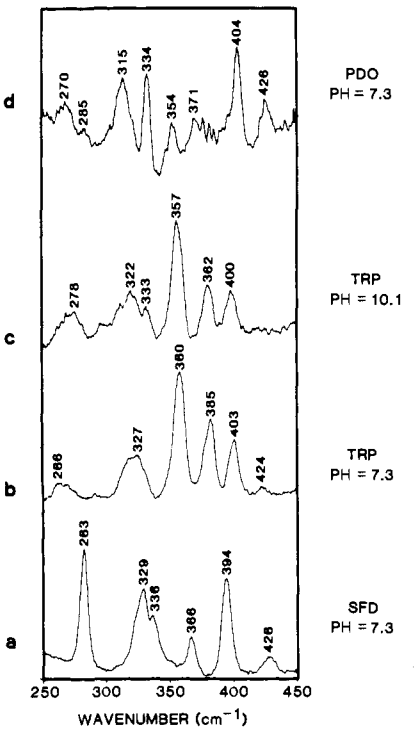

Figure 1. Resonance Raman spectra: (a) Oxidized spinach ferredoxin at pH 7.3 in HEPES [*N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid]. The spectrum was recorded with 457.9-nm excitation and 135-mW power and is an average of 53 scans. (b) Oxidized Rieske [2Fe-2S] protein from *T. thermophilus* at pH 7.3, HEPES. The spectrum was recorded with 488.0-nm excitation and 85 mW power and is the average of 40 scans. (c) Oxidized Rieske protein from *T. thermophilus* at pH 10.1, CAPS [(3-cyclohexylamino)-1-propanesulfonic acid]. The spectrum was recorded at 488.0-nm excitation and 110-mW power and is the average of 35 scans. (d) Phthalate dioxygenase from *P. cepacia* in HEPES, pH 7.3. The spectrum was recorded with 457.9-nm excitation and 135-mW power and is the average of 67 scans. Resonance Raman spectra of all the samples were recorded using an Ar^+ CW laser with a resolution of 4 cm^{-1} and a scan rate of 1 cm^{-1}/s. Scattered photons were collected by 135° backscattering off the surface of the frozen protein solution (74 K, ~800 mbar) (cf. Czernuszewicz and Johnson, ref 20). The spinach ferredoxin was purified by the method of Petering et al.[21] *T. thermophilus* Rieske protein was isolated by the method of Fee et al.[5] The protein had $A_{460}/A_{280} > 0.22$ before and after the experiment. Phthalate dioxygenase from *P. cepacia* was isolated by the procedure in ref 22. The A_{280}/A_{460} = 14 was used to check the purity of the sample before and after the experiment. The samples for resonance Raman experiments were prepared by passing the sample through a Sephadex G-25 column equilibrated with the buffer and concentrating it by slow evaporation under a stream of argon. The A_{460} of all the samples were >1/mm (i.e., >1 mM).

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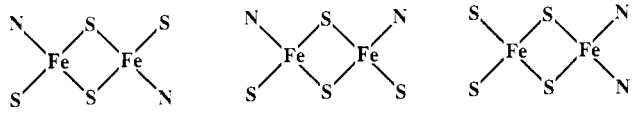
Chart I



a C_{2h} b C_{2v} c C_{2v}

proteins are above +100 mV. The latter are affected by a redox-linked protonation of the cluster wherein the oxidized form of the cluster reversibly loses a proton with $pK_a \sim 8$ (cf. ref 7 and references therein). We expect RR studies will contribute to an understanding of these important differences.

Resonance Raman spectra (250–450 cm^{-1}) of the oxidized forms of PDO, TRP at pH 10.1 and 7.3, and of SFD are presented in Figure 1. The RR spectrum of SFD is typical of $(Cys)_2FeS_2Fe(Cys)_2$-containing proteins. Characteristic features and proposed[8-11] assignments include Fe–S angle deformations below 200 cm^{-1}, a group of terminal Fe–S stretching motions between 330 and 370 cm^{-1}, the prominent symmetric Fe_2S_2 ring stretching mode near 390 cm^{-1}, and the peaks near 280 and 430 cm^{-1} ascribed[8] to ungerade (in centrosymmetric symmetry) Fe_2S_2 ring stretches. Model complexes mimicing the $(Cys)_2FeS_2Fe(Cys)_2$ clusters possess rigorous centrosymmetry[12] and the SFD cluster is at least approximately centrosymmetric (cf. ref 13). Recent polarized Raman studies[14] have shown, however, that the spectroscopically effective symmetry of the iron/sulfur core of SFD cannot be D_{2h} but is at most C_{2v} and possibly lower in symmetry. Nevertheless, the ungerade (in D_{2h}) motions, which are strictly Raman forbidden in the D_{2h} point group, are expected to remain weak in the absence of a substantial perturbation away from centrosymmetry. Therefore, the intensity of these peaks in the SFD spectrum is anomalously large if the assignments are correct. Such assignments are, however, complicated by stretch–bend interactions and by the fact that the terminal Fe–S–C and cysteine S–C–C angle bends are expected to be near 260 and 420 cm^{-1}, respectively. Studies of blue copper proteins[15] have shown that possible resonance enhancement of these angle bends cannot be discounted.

In addition to the above modes for ferredoxin-like iron–sulfur proteins, TRP and PDO are expected to exhibit Fe–N stretches (which, assuming histidine imidazole coordination to high-spin Fe(III), should be between 200 and 300 cm^{-1})[16-18] and other motions associated with Fe–N coordination. As expected, there are strong generic similarities between the RR spectra of ferredoxin-type proteins and Rieske-type proteins but also some striking differences (see Figure 1). The terminal Fe–S stretches between 330 and 370 cm^{-1} in SFD apparently persist (the analogous peaks appear at or below 360 cm^{-1} in TRP and PDO). In place of the single ring breathing mode near 390 cm^{-1} in SFD, however, there are at least two features in TRP, at ~380 and ~400 cm^{-1}, and in PDO, at ~370 and 404 cm^{-1}. In addition to the extra peak near 390 cm^{-1} in TRP and PDO, there are clearly two peaks near 270 cm^{-1} rather than one at 283 cm^{-1} as in SFD[19] and at least one additional peak below 200 cm^{-1} (not shown).

The appearance of extra peaks in the PDO and TRP spectra compared to SFD can be understood by considering the symmetry of $[Fe_2S^b_2]S^t_4$, where S^b is bridging sulfide and S^t is terminal mercaptide sulfur from cysteine, compared to the structural options of the iron–sulfur–nitrogen clusters. Assuming that the iron–ligand stoichiometry in TRP and PDO is $[Fe_2S^b_2]S^t_2N_2$ and that each Fe is tetrahedrally coordinated, three nominal structural symmetries structures are possible (Chart I). Two of these involve each iron having a $S^b_2S^tN$ coordination environment, the two possibilities differing in that one has a center of symmetry (structure a, nitrogen atoms pseudotrans across the cluster, point group C_{2h}) and the other does not (structure b, nitrogen atoms pseudocis, point group C_{2v}). The third possible structure (c) also has C_{2v} symmetry, but has both nitrogen atoms coordinated to the same iron; the latter structure is consistent with the Mössbauer results.[4]

In the point groups mentioned above, all vibrational modes are nondegenerate and therefore splitting of peaks by symmetry reduction cannot occur. In the centrosymmetric point groups (D_{2h} and C_{2h}), discounting possible contributions from skeletal modes of the ligands, there are nine possible Raman-active (gerade symmetry) vibrations. The RR spectrum of SFD shows only eight or nine peaks below 450 cm^{-1}; thus it is not necessary to invoke activation of ungerade motions in order to account for the observed Raman peaks. In TRP and PDO, however, there are at least 12 peaks in the RR spectra. If only cluster vibrations are being observed, the symmetry of these $[Fe_2S^b_2]S^t_2N_2$ clusters clearly cannot be centrosymmetric or nearly centrosymmetric (structure a). Of the two remaining structural choices, both C_{2v}, either with one nitrogen ligand on each iron (structure b) or with both nitrogen ligands on one iron (structure c), the RR data favor the latter structure. This is surmised because the observed number of peaks can only occur if the iron–sulfur core experiences a major perturbation away from centrosymmetry such that formerly ungerade motions become Raman allowed. This probably could not be accomplished by changing the symmetry of the cluster from nominally C_{2h} (N-trans) to C_{2v} (N-cis). Instead, the other C_{2v} structure (c), $S^t_2FeS^b_2FeN_2$, or a structure with still lower symmetry but with both nitrogens on the same iron atom, is probably required.

Finally, the relative RR intensities and some of the frequencies in TRP are pH dependent. This effect occurs over the same pH range that the previously noted changes in absorption spectrum and redox potential occur in TRP;[7] it is not observed in PDO or SFD. Distinct changes which occur on deprotonation are loss of the peak at 424 cm^{-1}, emergence of a new peak at 278 cm^{-1}, and the shift of the two peaks at 385 and 360 cm^{-1} to 382 and 357 cm^{-1}, respectively. These changes may be due to differing resonance enhancement arising from the observed pH dependence of the absorption spectrum or to actual changes in vibrational frequencies. Clearly, a major structural rearrangement does not accompany the ionization; the observed differences could result from deprotonation of a bound imidazole, as previously suggested.[7] More extensive studies of excitation profiles and isotope effects

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(particularly ^2H, ^{15}N, ^{34}S, and ^{54}Fe substitution) are required to clarify the reasons for this observation and to understand the differences between the TRP and PDO spectra. We are currently pursuing these studies.

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Registry No. PDO, 63626-44-8; N, 7727-37-9; L-Cys, 52-90-4.

Cyclopropane Stereomutation Catalyzed by One-Electron Oxidants

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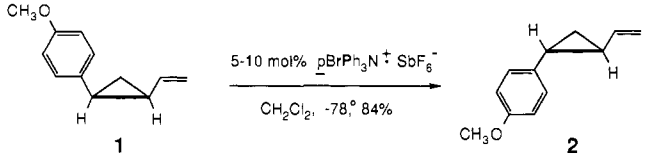
Cyclopropane cation radicals are proposed intermediates in the cis $\rightleftarrows$ trans isomerization of 1,2-diphenylcyclopropane under photooxidation conditions.[1,2] The cation radicals do not isomerize directly, however. Instead, chemically induced dynamic nuclear polarization (CIDNP) studies[2] reveal that back electron transfer from the sensitizer anion radical to the cyclopropane cation radical leads to the intermediate responsible for isomerization: a triplet 1,3-diphenyltrimethylene biradical. It was ultimately concluded that interconversion of the *cis*- and *trans*-1,2-diphenylcyclopropane cation radicals was slow even at room temperature.[2c,3]

*We have discovered that cis-1-p-anisyl-2-vinylcyclopropane (**1**) is rapidly isomerized by one-electron chemical oxidants at temperatures as low as −90 °C.* The thermodynamics of electron transfer exclude a biradical mechanism and isotopic labeling experiments bound considerably the mechanistic possibilities for stereomutation.



1[4] was isomerized to *trans*-cyclopropane **2** (84%) by 5–10 mol % *p*-$BrPh_3N^{\bullet+}SbF_6^-$ (**3**)[5] in CH_2Cl_2 at −78 °C or in CH_3CN at −40 °C (88%). The reaction was similarly catalyzed by $O_2^{\bullet+}SbF_6^-$ (**4**)[6] albeit in lower yield (48%). Above −78 °C in CH_2Cl_2, polymerization competed with isomerization using either catalyst but was effectively inhibited by the addition of 2,6-di-*tert*-butylpyridine, which had no effect on cyclopropane isomerization.

The isomerization half-life measured at −90 °C in CH_2Cl_2 with 10 mol % **3** was ≈10 min. A comparison with the extrapolated thermal cis → trans rearrangement rate of 1-phenyl-2-vinylcyclopropane[7] ($t_{1/2}^{-90\,°C} \approx 10^{25}$ min) reveals that the *p*-$BrPh_3N^{\bullet+}SbF_6^-$ catalyzed isomerization is accelerated by a factor of $\approx 10^{24}$!

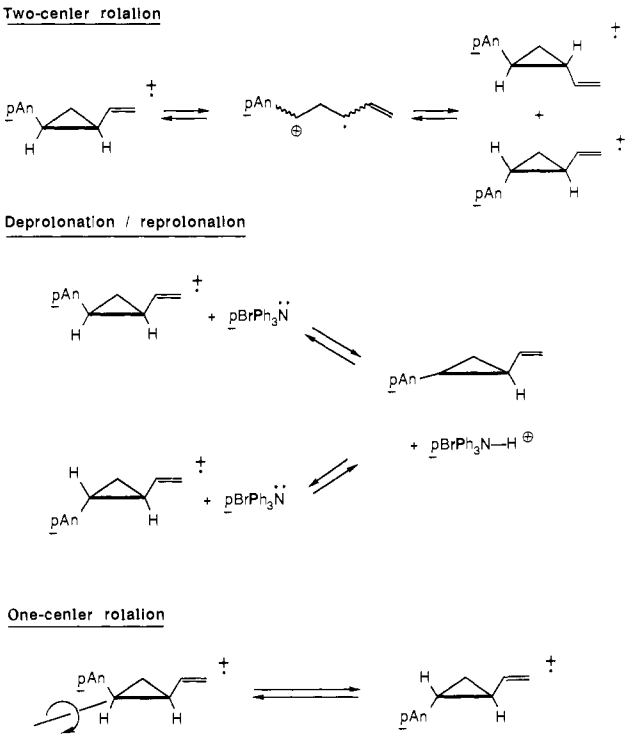
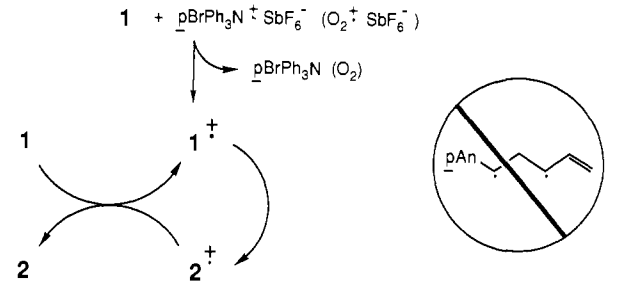


Figure 1. Three stereomutation mechanisms.

We propose a cation radical chain mechanism for isomerization based upon the one-electron oxidation chemistry of **3**[8] and **4**.[6] Excluded from this mechanism is the trimethylene biradical. The one-electron reduction of intermediate cyclopropane cation radicals by the strongest available reductant, *p*-$BrPh_3N$, does not provide enough energy to populate the biradical.[9] The energetics of electron transfer further permit **3** to be a reversible one-electron oxidant but not **4**.[10] Thus **4** can only serve as a chain initiator for isomerization. Shown below is a unified mechanism which utilizes both **4** and **3** in this fashion. The propagation steps then involve **1**, **2**, and their cation radicals.[11]



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(10) Reduction of $\mathbf{1}^{\bullet+}$ or $\mathbf{2}^{\bullet+}$ by O_2 would be endothermic by ca. 90 kcal/mol. This was calculated using the oxidation potentials of **1**[9] and **2**[9] and the calculated electrode potential for O_2 (5.3 V vs. SCE). The latter was obtained from Miller's empirical equation (Miller, L. L.; Nordblom, G. D.; Mayeda, E. A. *J. Org. Chem.* **1972**, *37*, 916), using the ionization potential of O_2 measured by photoelectron spectroscopy (12.08 eV: Collin, J. E.; Delwiche, J.; Natalis, P. *Int. J. Mass Spectrom. Ion Phys.* **1971**, *7*, 19) and converting the Ag/Ag^+ potential so obtained to the SCE potential by adding 0.34 V.

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