Supplementary Material Available: General description for the preparation of compounds, elemental analyses, and figures showing a different perspective to that in Figure 2 and part of the EXSY spectrum of (*S*,*R*,*R*,*S*)-LPt(5'-GMP)$_2$ (3 pages). Ordering information is given on any current masthead page.

Dithiolene Coordination in the Molybdopterin Cofactor of DMSO Reductase: In Situ Evidence from Resonance Raman Spectroscopy

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We present resonance Raman (RR) spectra of DMSO reductase (DR) from *Rhodobacter sphaeroides*[1,2] which strongly support the view[3-6] that its Mo atom is bound to pterin via a dithiolene chelate. DMSO reductase is a member of a class of redox enzymes that contain an extractable molybdopterin cofactor that is able to reconstitute nitrate reductase activity in a cofactor-minus mutant, Nit-1, of *Neurospora crassa*.[7] The extracted cofactor is labile and has so far eluded direct structural characterization.[6] On the basis of persuasive chemical evidence, however, the structure shown in Figure 1 was proposed for the Mo cofactor in hepatic sulfite oxidase and milk xanthine oxidase. The major features of the structure of molybdopterin were confirmed by structural studies on the carboxamidomethyl derivative.[6] More recently, the Mo cofactor of DR has been shown to contain an extended form of molybdopterin, molybdopterin guanine dinucleotide, in which the pterin is attached to 5'-GMP through a pyrophosphate linkage.[8] DR is an attractive vehicle for in situ characterization of the cofactor by RR spectroscopy, because of its rich electronic spectrum, also shown in Figure 1, which is unobscured by other chromophores, i.e., Fe–S clusters and flavin, or heme, that are present in most Mo enzymes.[9] Reported RR spectra of xanthine oxidase are dominated by Fe–S cluster modes.[10]

Figure 2 shows a portion of the high-frequency RR spectra of DR in oxidized [DRox] and reduced [DRred] form, excited at 676.4 nm. This wavelength is near the lowest energy electronic absorption bands, 720 and 640 nm, for DRox and DRred. The RR band at 1575 cm^{-1} in DRox and 1568 cm^{-1} in DRred is in the C=C stretching region. Its assignment to the dithiolene C=C stretch is supported by the down shift upon reduction from Mo(VI) to Mo(IV), due to redistribution of electron density in the dithiolene ring. The direction of the redox-associated frequency shift is not easily predictable. The C=C bond order should be decreased by electron donation from the dithiolene π system to the metal. For Mo(IV) the C=C bond order may also be decreased by back-donation from the metal d_π to the dithiolene. The net effect is to produce a slight (7 cm^{-1}) down shift in ν(C=C) upon reduction. The frequency is higher in DRred than it is in the oxo-Mo(IV) bis-dithiolene complex, $MoO(S_2C_2(CO_2Me)_2)_2^{2-}$,[11] 1535 cm^{-1}, possibly reflecting the increased competition between two dithiolene ligands for the Mo(IV) acceptor d_π orbitals.

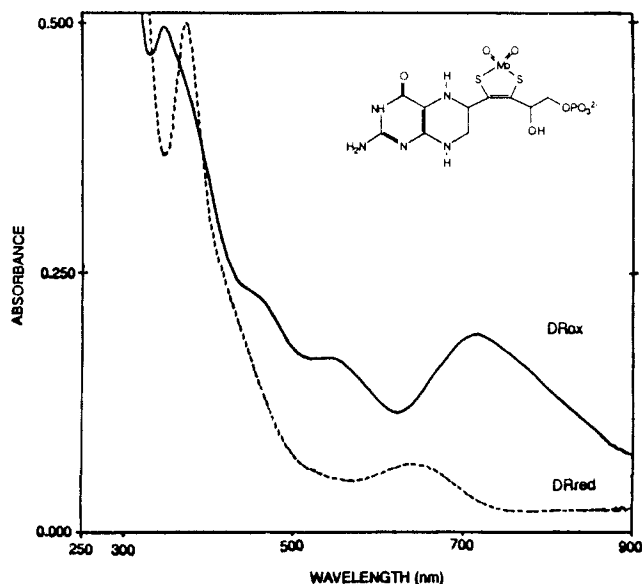

Figure 1. Absorption spectra of DR (7.9 mg/mL, 0.096 mM) prepared from *Rh. sphaeroides* by a modification[2] of the method of Satoh and Kurihara.[1] The enzyme as isolated was in the oxidized form, Mo(VI), and was reduced with sodium dithionite. Excess dithionite was removed by anaerobic gel (PD-10) chromatography. The enzyme appeared as a single band on SDS-PAGE gels. Its activity (16 μmol of DMSO oxidized/(min·mg)) was established by monitoring the substrate-dependent oxidation of dithionite-reduced benzyl viologen in 50 mM Tris·HCl, pH = 7.5. The structure of the molybdopterin moiety of molybdopterin guanine dinucleotide, which is proposed[6] on the basis of chemical evidence, is shown in the inset.

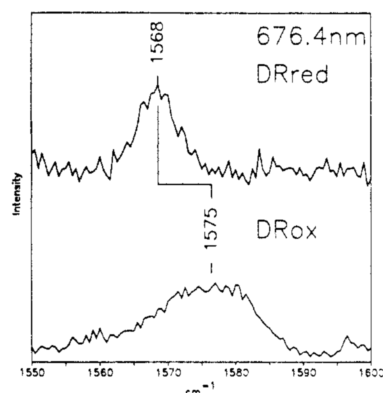

Figure 2. RR spectrum, 676.4-nm excited, of oxidized and reduced DR (0.91 mM) in the C=C stretching region. The spectra were obtained from frozen solution on a cold finger cooled to 77 K.[18] DRred was loaded onto the sample cell in an O$_2$-free glove box. Excitation was from a Kr$^+$ laser. The scattered light was scanned with a Spex 1401 double monochromator equipped with photon counting electronics. Conditions: 200-mW output laser power; 6-cm^{-1} slit width; 0.5-cm^{-1} steps; 1 s/step.

Figure 3 shows RR spectra in the 200–500-cm^{-1} region, where Mo–ligand stretching modes are expected. Comparison of the spectra with those of enzyme extracted from bacteria grown on ^{34}S shows isotope-sensitive bands, which are attributable to Mo–S stretching. DRred shows two ^{34}S-sensitive bands, at 352 and 383 cm^{-1}, and so does DRox, at 350 and 370 cm^{-1}. In addition, the DRox bands have shoulders at 336 and 377 cm^{-1}, which also appear to shift on ^{34}S substitution, as do the weak 368-, 417-, and

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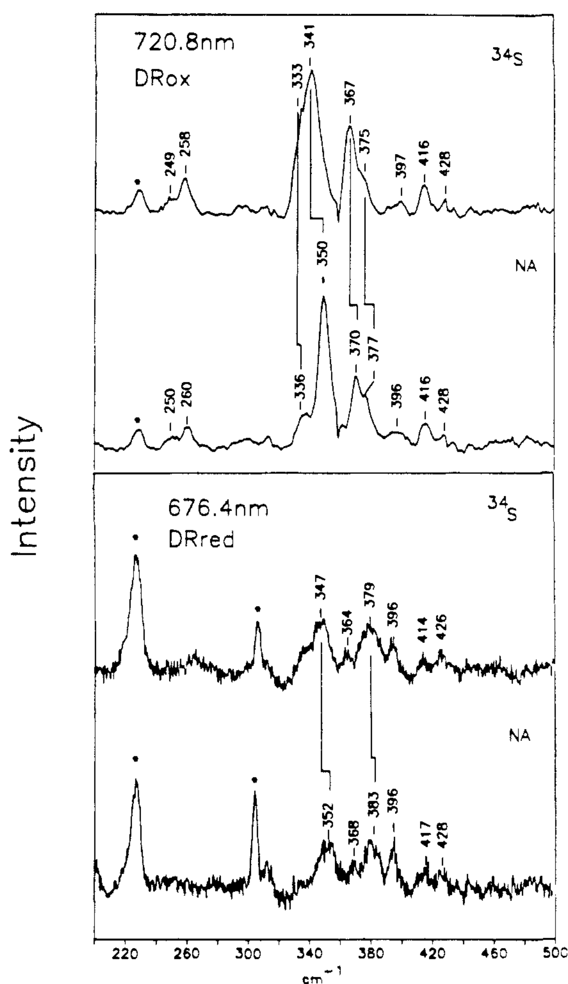

Figure 3. Low-frequency RR spectra, showing ^{34}S shifts of bands having Mo-S stretching character in oxidized (top) and reduced (bottom) DR. Excitation at 720.8 nm produces stronger enhancement for DRox, although the same features can be seen with 676.4-nm excitation. The spectrum of DRox was obtained by 180° backscattering from a frozen solution mounted on a cold finger cooled to 77 K. The 720.8-nm excitation line was provided by an Ar^+ pumped dye laser (Pyridine 2). The scattered light was collected with a Spex Triplemate equipped with a CCD (Photometrics). Conditions: 100-mW output laser power. The spectrum of DRred was obtained as described in Figure 2. Peaks marked with an asterisk are due to ice (228 cm^{-1}) or laser plasma lines (304 cm^{-1}). The ^{34}S-containing protein was obtained from *Rb. sphaeroides* grown on ^{34}S. ^{34}S, 90 atom %, obtained from Isotec, Inc., as elemental S was converted to $(NH_4)_2{}^{34}SO_4$ by boiling in a 3:1 mixture of HCl and HNO_3 in an oil bath at 170 °C until no more yellow fumes were seen. The solution was neutralized with NH_4OH and dried. Cultures of *Rb. sphaeroides* were grown for three generations on sulfate-free medium to deplete endogenous sulfur. The culture was then transferred to $^{34}SO_4$-containing medium for growth and isolation of ^{34}S-enriched DR.

428-cm^{-1} bands of DRred, but improved spectra are needed to confirm these shifts. (For DRox stronger enhancement was obtained at 720.8 nm, directly in resonance with the 720-nm absorption band.) The two Mo-S bonds of a dithiolene chelate should give rise to two stretching modes and can account for the two main ^{34}S-sensitive bands. The frequencies are nearly the same in the two oxidation states, but are slightly lower for DRox than for DRred, consistent with the slightly higher C=C frequency. These frequencies are expected to correlate negatively on electronic grounds.[12] Mo^{IV}-S frequencies have been reported in the 350-390-cm^{-1} range for various dithiolene complexes.[11,13] The weaker candidate ^{34}S-sensitive bands might arise from Mo-S bonds if the Mo is bound to the protein via cysteine ligands. EXAFS spectra of other molybdopterin proteins have indicated Mo-S bonds[14-17] in excess of two. Alternatively, the additional bands may arise from ligand deformation coordinates, e.g., SCC bending, coupled to the Mo-S stretches.

In summary, RR spectra, in resonance with the red absorption bands of DMSO reductase, reveal the presence of C=C stretching and Mo-S stretching vibrational bands, thereby confirming the presence of a dithiolene chelate structure in the molybdenum cofactor.

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Microviridin: A Novel Tricyclic Depsipeptide from the Toxic Cyanobacterium *Microcystis viridis*

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Cyanobacteria of *Microcystis* species have been extensively studied from the environmental, toxicological, biological, and chemical points of view because they are responsible for water blooms that frequently produce potent hepatotoxins. These toxins are classified as microcystins and are considered to be harmful to the health of humans, cattle, and wild animals.[1] During our work on the toxins,[2] we found that an HPLC fraction contained a peptide, which was different from microcystins, and exhibited tyrosinase inhibitory activity. We herein describe elucidation of its unusual structure.

An axenical clonal strain of *M. viridis* (NIES-102),[3] isolated from a bloom on Kasumigaura Lake, was cultivated in MA medium, and the dried cells (100 g) were extracted with methanol. The methanol extract was subjected to HPLC [Ultron C_{18}, CH_3CN/H_2O (25:75)], and a peak preceding the one containing microcystins LR + YR was collected and further purified by using a TSK gel DEAE-2SW column [20 mM phosphate buffer (pH 7.0) containing 0.3 M NH_4OAc] to afford a colorless solid, microviridin (87 mg).

Microviridin (1) [(Chart I); $[\alpha]_D$ +21.7° (c 0.95, MeOH); λ_{max} 220 (ϵ 57 000), 278 nm (ϵ 8800); $C_{85}H_{100}N_{16}O_{24}$ [HRFABMS m/z 1729.7220 $(M + 1)^+$]; negative in a ninhydrin test] produced 14 amino acids, Asp, Thr, Ser, 2 × Glu, 2 × Gly, 3 × Tyr, Phe, Lys, Trp, and Pro, on hydrolysis [TsOH/3-(2-aminoethyl)-indole/H_2O, 110 °C, 22 h].[4] The GCMS analysis using a chiral

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