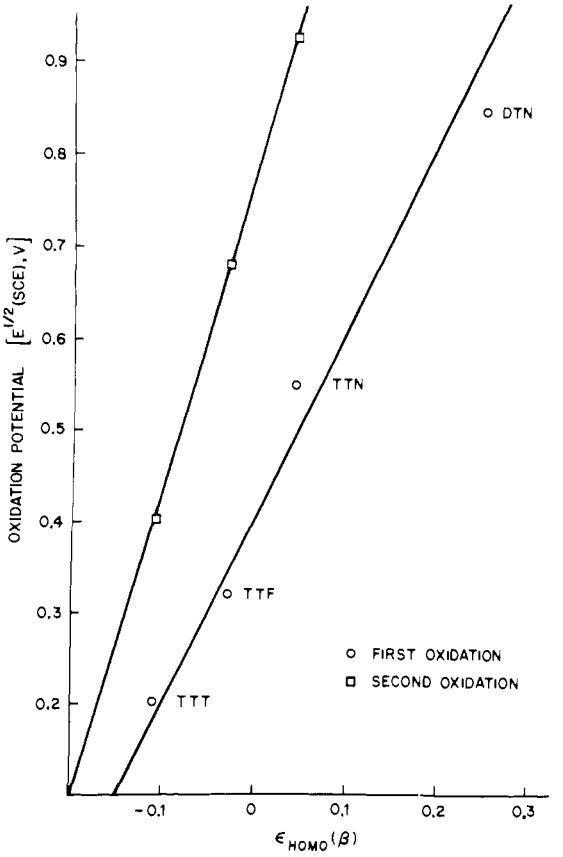

Figure 2. A plot of oxidation potentials (vs. SCE in acetonitrile with n-Bu_4NBF_4 as supporting electrolyte) against the calculated energy of the highest occupied molecular orbital.

carbons 1, 2, and 3 as shown in Table I and symmetry related atoms). These results are consistent with the higher dimensionality observed in certain TTT and TST (tetraselenatetracene) salts[19] in which the interstack sulfur (or selenium) distances are short and spin may be delocalized across stacks as well as along stacks.

Acknowledgments. We thank S. H. Glarum and D. J. Freed for the use of ESR equipment and for mass spectral analysis of TTA. Also, we thank E. M. Engler of IBM for helpful discussions. F. B. B. acknowledges partial support from the National Science Foundation in the form of Grant No. SMI-077.

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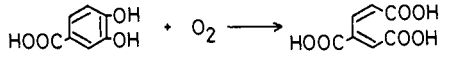
Received February 16, 1978

Resonance Raman Spectra of Protocatechuate 3,4-Dioxygenase. Evidence for Coordination of Tyrosine Residue to Ferric Iron

Sir:

We wish to report the first well-resolved resonance Raman spectra of a nonheme iron containing dioxygenase, protocatechuate 3,4-dioxygenase (protocatechuate:oxygen 3,4-oxidoreductase, EC 1.13.11.3). The spectroscopic evidence presented in this paper indicates that the tyrosine residue coordinates to the ferric iron, a sole cofactor of the enzyme. This enzyme, isolated from the bacterium, *Pseudomonas aeruginosa*, catalyzes the intradiol cleavage of protocatechuic acid with the insertion of two atoms of molecular oxygen to form β-carboxy-*cis,cis*-muconic acid[1] (eq 1). This enzyme contains



(1)

eight atoms of ferric iron per molecule (mol wt 700 000) and consists of eight identical protomers, each of which is composed of two pairs of nonidentical subunits ($\alpha_2\beta_2$).[2] The enzyme shows a red color with a broad absorption near 450 nm which is attributable to the ferric iron coordinated with some amino acid residues of the polypeptide chains (see the inset of Figure 1). When the substrate (protocatechuic acid) is added anaerobically, the visible spectrum shows an increase in intensity of ~480 nm implying the formation of an enzyme-substrate (ES) complex. Upon admission of oxygen a new absorption arises at 520 nm, suggestive of the formation of a ternary complex (ESO_2). When the substrate is consumed, the original spectrum is recovered.[3] Apparently the iron atoms play an important role in the activation of oxygen and/or substrate. Elucidation of the coordination environment around the ferric iron is thus of primary importance for understanding the mechanism of the enzymic reaction. A previous report suggested, on the basis of EPR spectroscopy, a tetrahedral arrangement of four cysteinyl sulfur atoms similar to the case of rubredoxin,[4] while another study based on Mössbauer spectrum claimed coordination of oxygen or nitrogen, but not sulfur, to the ferric iron.[5] Thus their conclusions are inconsistent with each other. As the resonance Raman scattering

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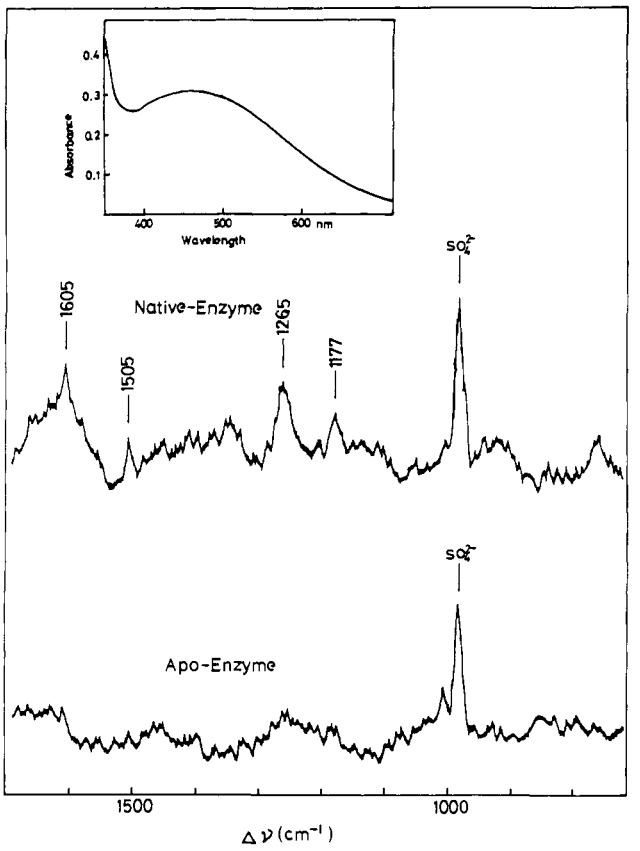

Figure 1. Raman spectra of protocatechuate 3,4-dioxygenase. Instrumental conditions: excitation, 488.0-nm line of Ar^+ laser; power, 70–140 mW at a sample point; time constant, 16 s; slit width, 180 μm; scan speed, 10 cm^{-1}/min; temperature, 15 °C. Concentrations: 32 and 29 mg/mL for the native and apo enzyme, respectively, in 50 mM of Tris-acetate buffer, pH 8.5. The inset shows the visible absorption spectrum of protocatechuate 3,4-dioxygenase, 23.3 mg, in 2.8 mL of 50 mM Tris-acetate buffer, pH 8.5.

technique is known to give structural information on chromophores,[6,7] we applied it to this enzyme.

The crystalline native enzyme and its apo enzyme were prepared as described previously.[8] As the intense visible absorption band at ~450 nm (ϵ 2.6 × 10^4 M^{-1} cm^{-1}) is associated with a charge transfer from the ligand to metal, a ligand-iron stretching mode or some internal vibrations of the ligand may gain resonance Raman intensity upon excitation at wavelength near the 450-nm band. Thus the Raman spectra were excited by the 488.0-nm line of an argon ion laser (Spectra Physica Model 164) and were recorded on a JEOL-400D Raman spectrometer equipped with HTV-R649 photomultiplier.

Figure 1 shows the resonance Raman spectra of the native and apo enzyme in the presence of 1% $(NH_4)_2SO_4$ as an internal reference. Four prominent Raman lines were observed at 1177, 1265, 1505, and 1605 cm^{-1} for the native enzyme, but none of these lines was detectable for the apo enzyme and the colorless ferrous form of enzyme prepared from the native enzyme by reducing with $Na_2S_2O_4$ under anaerobic conditions. Thus, the appearance of these Raman lines apparently requires the presence of ferric iron. These lines appear to be in resonance with the visible absorption band, and are presumably due to the internal vibrations of the coordinated amino acid residue. Their frequencies were unaltered in D_2O solution, indicating that the residues involved in the appearance of the Raman lines contain no replaceable hydrogen. The Raman spectrum of *p*-cresol-iron(III) complex prepared by mixing ferric ammonium sulfate with *p*-cresol at pH 7.0 showed four lines at 1180, 1222, 1488, and 1618 cm^{-1}. *p*-Cresol in 1 M NaOH solution also gave the corresponding Raman lines at 1176, 1276, 1490, and 1607 cm^{-1} with relative intensities different from those of the enzyme. These spectral data resemble those of iron(III)-transferrin reported by Gaber et al.,[9] which show four prominent Raman lines at 1174, 1288, 1508, and 1613 cm^{-1}. They assigned those lines to the vibration of phenolate ion of the coordinated tyrosine residue, based on study of a bis phenolate-iron(III) complex.

The present Raman spectrum is thus explicable in terms of the internal vibration of a coordinated phenolate anion of tyrosine residue. The four characteristic Raman lines of the native enzyme remained unshifted in the ES complex though with different relative intensities. This suggests a little conformational change at the active site and retention of the coordinated tyrosine upon substrate binding. Upon coordination of a cysteinyl sulfur to Fe^{3+} ion the Raman line due to Fe^{3+}–S stretching modes is expected to appear in a region between 250 and 350 cm^{-1} as seen in iron-sulfur proteins.[10] Despite a careful search, the corresponding line was not observed. This does not, however, warrant the conclusion that cysteinyl sulfur is not coordinated to the ferric iron of this enzyme.

The present work thus provides an example of successful application of the resonance Raman spectroscopy to the structural studies of such a giant molecule as nonheme iron containing dioxygenases. Further detailed studies of several nonheme iron containing dioxygenases are in progress.

Acknowledgment. This work has been supported in part by a grant from the Naito Foundation and by a Grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture, Japan.

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Catalytic Hydrolysis of Phenyl Esters in Aqueous Didodecyldimethylammonium Vesicles: Remarkable Rate Difference between Intra- and Intervesicle Reactions

Sir:

Stable, well-organized aggregates are formed in water from a variety of dialkylammonium and related compounds.[1-3]

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