$(CH_3)_3SiOSO_2^-$ does not react with ethanol or methanol

$$(CH_3)_3SiOSO_2^- + ROH \not\rightarrow (CH_3)_3SiOH + RSO_3^-$$

verifying that silanol is a stronger acid than either ethanol or methanol.

The slow reaction of the sulfite in Me_2SO is also of interest. In Me_2SO solvolysis is unlikely and the observed reaction must occur via another process. One such route is suggested by the aqueous chemistry of ammonia and sulfur dioxide. NH_3 and SO_2 are known to form $(NH_4)_2SO_3$ in the presence of water.[18] If an equilibrium exists in solution between the $NH_4(CH_3)_3SiOSO_2$ and its molecular components NH_3, SO_2, $(CH_3)_3SiOH$, $((CH_3)_2Si)_2O$, and H_2O, the appropriate reactants are available to form the observed precipitate. While silanol ordinarily reacts with itself very slowly to form $((CH_3)_3Si)_2O$ and H_2O, the reaction is extremely condition dependent and is strongly sensitive to surface conditioning of glassware.[19] Under conditions where siloxane is formed from silanol the following reactions are consistent with the observed decomposition products.

$$2(CH_3)_3SiOH \rightarrow ((CH_3)_3Si)_2O + H_2O \quad (8)$$

$$H_2O + 2NH_3 + SO_2 \rightarrow (NH_4)_2SO_3(s) \quad (9)$$

The conclusions drawn here confirm the reaction scheme postulated in our earlier paper on this unique reaction.[1] Furthermore, the identification of the solid product from the reaction now provides us with definitive knowledge of the overall reaction stoichiometry.

$$4((CH_3)_3Si)_2NH(l) + 4SO_2(l) \rightarrow 3(CH_3)_3SiNSO(l) + 2((CH_3)_3Si)_2O(l) + NH_4(CH_3)_3SiOSO_2(s)$$

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(19) Licht, V. K.; Kriegsmann, H. *Z. Anorg. Allg. Chem.* **1963**, *323*, 252.
(20) Nakamoto, K. "Infrared and Raman Spectra of Inorganic and Coordination Compounds", 3rd ed.; Wiley: New York, 1978.

Resonance Raman Studies of Ferric NADH Transients

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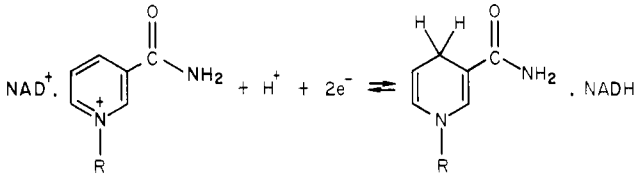
Contribution from the Department of Chemistry, Clarkson College of Technology, Potsdam, New York 13676. *Received February 23, 1981. Revised Manuscript Received May 11, 1981*

Abstract: Resonance Raman spectra of blue and red-violet ferric nicotinamide adenine dinucleotide transients were measured by excitation into absorption bands at 540 and 375 nm. Intense Raman bands observed at 1547 and 1521 cm^{-1} for NADH and its blue ferric transient, respectively, were assigned to nicotinamide ring stretching vibrations. The 26-cm^{-1} lowering of the band position in the transient is related to a decrease in ring bond orders either by complexation to ferric ions or by formation of a half-oxidized free radical intermediate. Resonance enhancement of amide I and nicotinamide ring vibrational modes with excitation into the 375-nm absorption band was linked to differences in the nature of the two excited states.

Nicotinamide adenine dinucleotide (NAD) is a coenzyme that occurs widely in respiratory and photosynthetic systems as a major electron carrier. It is composed of two bases, nicotinamide (3-pyridine carboxamide) and adenine, linked by two sugars and two phosphate groups. The basis of the biological activity of NAD lies in the ability of the pyridine ring to undergo two-electron transfer



At the oxidizing end of the respiratory chain of most organisms, two electrons are transferred from NADH to NADH dehydrogenase,[1] a protein which contains nonheme iron, acid labile sulfur, and flavin mononucleotide (FMN). Since flavins are known to undergo two reversible one-electron reductions, NADH dehydrogenase[2] is believed to be the site at which the electron flow changes from simultaneous two-electron transfer to sequential one-electron transfer. The mechanism by which electrons are transferred from NADH to NADH dehydrogenase is uncertain. The reaction of FMN and NADH in neutral aqueous solution produced absorption spectra[3] characteristic of the flavin free radical, indicating that the flavin functioned as a one-electron acceptor. There was no evidence to show the formation of an NADH free radical species. The addition of sodium nitrosopentacyanoferrate(III) to the FMN–NADH mixture produced a complicated reaction, the net result of which was the oxidation of NADH to NAD^+ and reduction of the iron(III) complex. The flavin acted as an intermediary but underwent no overall change.

The direct reaction of NADH with iron(III) has also been investigated.[4,5] When NADH is mixed with ferric perchlorate at pH 3, two short-lived (half-life of several seconds) blue complexes with stoichiometries Fe(NADH) and $Fe(NADH)_2$ were formed. Both have electronic absorption bands at 540 nm (ϵ = 900) and 375 nm (ϵ = 6000). Breakup of the complex produces Fe(II) and NAD^+ which do not absorb intensely in the visible spectrum, and no complex is formed by mixing NAD^+ with iron(II) or iron(III). Since iron(III) phosphate and iron(III)

(1) King, T. E.; Howard, R. L.; Kettman, J., Jr.; Hedgekar, B. M.; Kuboyama, M.; Nickel, K. S.; Possehl, A. E. *BBA Libr.* **1966**, *8*, 441.
(2) Beinert, H.; Palmer, G. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1965**, *27*, 105.
(3) Mayhew, S. G.; Ludwig, M. L. *Enzymes, 3rd Ed.* **1975**, *12B*, 57-118.
(4) Gutman, M.; Margalit, R.; Schejter, A. *Biochemistry* **1968**, *7*, 2778, 2786.
(5) Gutman, M.; Eisenbach, M. *Biochemistry* **1973**, *12*, 2314.

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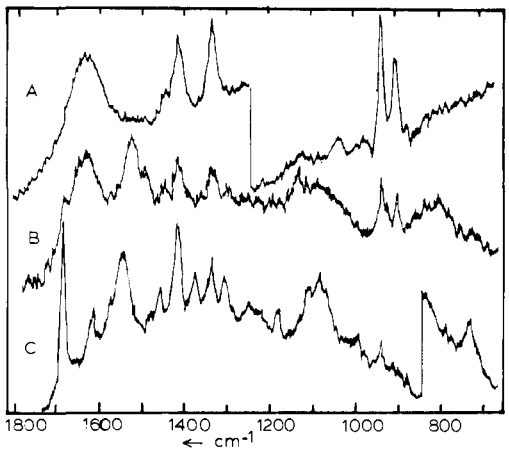
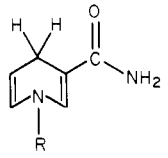


	sensitivity, counts/s	spectral slit width, cm^{-1}	time constant, s	scan speed, cm^{-1}/s
A	2000	10	2	1.0
B	1000	8.7	2	1.0
C	4000	8.7	5	0.5

Figure 1. Raman spectra of the following: A, 0.005 74 M ferric perchlorate hexahydrate in 0.2 M glycine, pH 7.5; B, 1:1 mixture of solution in A and 0.0111 M NADH in 1 mM sodium bicarbonate, pH 7.8; C, 0.0700 M NADH in 1 mM sodium bicarbonate, pH 7.8. Experimental conditions: excitation wavelength, 514.5 nm; laser power, 250 mW.

adenosine triphosphate complexes do not show intense visible absorption, this mode of binding can be ruled out as the primary source of color. Nitrogen atoms of the nicotinamide group are ineffective as donor atoms to the metal since the nicotinamide ring tertiary N is a very weak base and the amide N is a poor metal binder that is susceptible to hydrolysis. If the structure of the ring were bent



π-complex formation through the two olefinic groups is a viable alternative. A planar ring with π-delocalization through the ring and amide group might also form a π-complex with Fe(III). This π-type interaction needs to be experimentally confirmed since energy and electron transfer between NAD(H) and its immediate donor or acceptor appears to proceed via a π-interaction pathway.

Herein, we report the resonance Raman spectrum of a longer lived red-violet Fe^{III}NADH complex that is formed in basic solution and the short-lived blue complex in acidic solution as well as the time dependence of the latter spectra.

Experimental Section

For basic solutions, β-NADH, disodium salt (grade III, Sigma) was dissolved in 1 mM sodium bicarbonate (pH 7.8) to give a 0.0111 M solution and was mixed 1:1 with 0.005 74 M ferric perchlorate in 0.2 M glycine solution (pH 7.5). A red-violet solution resulted, and a small amount of ferric hydroxide precipitate was allowed to settle before using the supernatant solution. For acidic solutions, 0.0200 M β-NADH, disodium salt in bicarbonate solution (pH 9.4), was mixed 1:1 with 0.0110 M ferric perchlorate in 0.1 M glycine buffer (pH 2.0). A blue solution resulted upon rapid mixing of the two solutions. Over a period of 0.5 h this solution turned from blue to red-violet to dark red.

Raman spectra with Spectra-Physics Model 164 Ar^+ laser excitation were measured with a Jarrell-Ash 25-100 double monochromator using an RCA C31034 phototube for detection. Spectra in the ultraviolet region were recorded with a Spex 0.5-m double monochromator using a Gencom picoammeter for DC detection and a Coherent Model CR-3000K laser for excitation. Solutions were mixed (or recirculated) with an LKB peristaltic pump through a T junction and mixing chamber and then through quartz tubing (~1-mm diameter) arranged at 90° to the laser beam and monochromator slits. Scattered light was collected at 90° to the incident beam.

Table I. Raman Band Frequencies (cm^{-1}) from Figure 1 and Vibrational Assignments

spectrum A ferric glycinate	spectrum C, NADH	spectrum B, Fe(III) gly–NADH mixture	tentative band assignt[a]
	1686 (vs)	1686 (w)	amide I
1636 (s, br)			$\nu_a(COO^-)$, gly
		1628 (s, br)	amide I, NAD^+
	1614 (mw)		amide II
	1547 (s, br)	1521 (s, br)	$\nu(C{=}C) + \nu(C{=}N)$
		1488 (w, sh)	$\nu(C{=}C) + \nu(C{=}N)$
	1451 (w)		ν(adenine)
1442 (w, sh)			$\rho_s(CH_2)$, gly
	1417 (s)		ν(adenine)
1414 (s)		1417 (m)	$\nu_s(COO^-)$, gly
	1374 (mw)		ν(adenine)
	1337 (m)		ν(adenine)
1331 (s)		1331 (m, br)	$\rho_w(CH_2)$, gly
	1307		ν(adenine)
		1293 (vw)	
	1172 (mw)		
		1126 (mw)	
	1112 (mw, sh)		$\nu(PO_2)$, phospho-diester
	1083 (m, br)		
1033 (w, br)			ν_a(CNN), gly
	938 (w)		
934 (vs)		934 (s)	$\nu_4(ClO_4^-)$
898 (s)		898 (ms)	ν_s(CCN), gly
	730 (m)		ν(adenine)

[a] Bands are assigned to nicotinamide group unless otherwise noted.

Results and Discussion

Raman spectra of aqueous ferric glycinate and β-NADH disodium salt solutions and a 1:1 mixture (~1:2 Fe^{3+}:NADH molar ratio) absorbing maximally at 540 nm are illustrated in Figure 1 for the 1800–700-cm^{-1} region. An 0.0700 M NADH solution Raman spectrum is shown for its superior signal-to-noise ratio. Spectra of an 0.0111 M NADH solution showed no difference in relative band intensities or positions. Raman band maxima, their relative intensities, and tentative vibrational mode assignments are presented in Table I.

In the ferric glycinate spectrum (A, Figure 1) all the Raman bands originate from glycinate[6] except the band at 934 cm^{-1}, which is assigned to the totally symmetric perchlorate ion stretching mode. In NADH the nicotinamide and adenine moieties show very little vibrational interaction even though the π-clouds of the two rings interact with each other. Therefore, Raman spectra of adenine and adenosine monophosphate, which have been well studied,[7,8] were used to assign adenine bands in spectrum C, Figure 1. The phosphodiester groups show broad bands near 1100 cm^{-1}, while bands from the sugar residues are too weak to be detected.

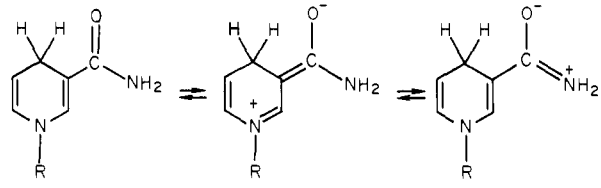
Raman bands at 1686 (vs) and 1614 (mw) cm^{-1} of NADH are present either very weakly or not at all in the spectrum of the Fe(III)–NADH mixture. Since only a single carbonyl group occurs in NADH, these bands are assignable to amide I and amide II modes of the primary amide[9] substituent of the nicotinamide ring. Their low intensity in the mixture Raman spectrum may indicate that Fe(III)-catalyzed hydrolysis of the amide group has occurred to form a carboxylate group or that amide vibrations were not resonance enhanced with 514.5-nm excitation into the

(6) Nakamoto, K. "Infrared and Raman Spectra of Inorganic and Coordination Compounds", 3rd ed.; Wiley-Interscience: New York, 1978.
(7) Lord, R. C.; Thomas, G. J. *Spectrochim. Acta, Part A* **1967**, *23A*, 2551.
(8) Nishimura, Y.; Hirakawa, A. Y.; Tsuboi, M. In "Advances in Infrared and Raman Spectroscopy"; Clark, R. J. H., Hester, R. E., Eds.; Heyden: London, 1978; Vol. 5, p 217.
(9) Dollish, F. R.; Fateley, W. G.; Bentley, F. F. "Characteristic Raman Frequencies of Organic Compounds"; Wiley-Interscience: New York, 1974.

electronic absorption band of the blue ferric NADH transient. As no new Raman bands or additional Raman band intensity assignable to a symmetric carboxylate stretching mode in the 1400-cm^{-1} vicinity are apparent if spectrum B is compared with spectra A and C in Figure 1 (ionized carboxylate groups show $\nu_s(COO^-)$ near 1415 cm^{-1}), we prefer the latter reasoning. By comparing the relative intensities of ferric glycinate bands at 934, 898, and 1414 cm^{-1} in spectrum A to the same bands in spectrum B, we conclude that most if not all of the Raman band intensity at 1417 cm^{-1} in spectrum B originates from the glycinate, $\nu_s(COO^-)$, mode.

Neither glycinate nor adenine, adenosine, and adenosine monophosphate (AMP)[7,8] show significant Raman band intensity in the 1500–1600-cm^{-1} range; therefore, the strong 1547- and 1521-cm^{-1} bands of NADH and the Fe^{III}NADH transient, respectively, are assigned to $\nu_s(C{=}C) + \nu_s(C{=}N)$ modes of the reduced nicotinamide ring. To account for the lower frequency of this band in the blue transient, we suggested two possibilities. The nicotinamide ring in NADH has been converted to a free radical species in the blue complex (nicotinamide derivatives have been converted to free radical species[10] which absorb at 647 nm), and the iron is not complexed to the ring. The removal of an electron from the relevant nicotinamide ring π-bonding orbital would decrease the overall bond orders in the ring and thus lower the $\nu_s(C{=}C) + \nu_s(C{=}N)$ band frequency. However, the only electron acceptor in the system is ferric ion, and no evidence of ferrous ion was found in the initial stages of the reaction when the blue transient is formed.[5]

The most likely possibility is therefore a π-complex between iron(III) and the nicotinamide ring. This ring in NADH is probably planar and is electronically π-delocalized due to the contribution of several resonance forms.[11,12]



Nevertheless, much of the π-electron density should be delocalized in the two C=C bonds in the ground electronic state.

Olefins are known to act as π-acid ligands in which the olefin π-bonding orbitals interact with σ-metal orbitals and metal d_π orbitals interact with empty ligand π^* orbitals. The increased electron density in the π^* orbitals of the ligand causes a decrease in the C=C stretching frequency. Several π-acid metal complexes have been studied by infrared and Raman spectroscopy. Nonconjugated olefins such as norbornadiene, NBD,[13,14] and 1,5-hexadiene and conjugated olefins such as butadiene[16,17] and cyclooctatetraene, COD,[18] coordinate to metal through two C=C double bonds, which are also available for metal binding in the reduced nicotinamide moiety. Free ligand ν(C=C) bands were observed in the 1550–1650-cm^{-1} region whereas coordinated ligand ν(C=C) bands occurred at lower frequency between 1400 and 1500 cm^{-1}. Substantial Fe(III)–(reduced nicotinamide ring) bonding appears to be indicated by the 26 cm^{-1} lowering of the $\nu_s(C{=}C) + \nu_s(C{=}N)$ band in the Fe^{III}NADH complex. Metals that were involved in complexation with COT and NBD were all "soft" metals. In contrast, ferric ion is "hard" with a small, dense electron cloud, with which overlap with the π-orbitals of the nicotinamide ring in NADH is expected to be poor. The comparatively small frequency shift may reflect the weak bonding and consequent instability of the complex.

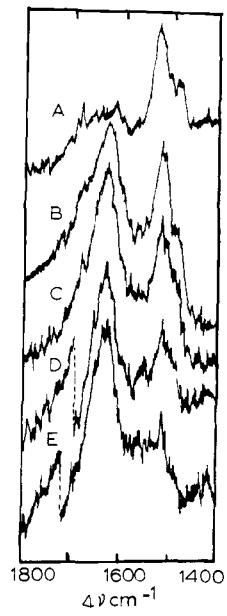


Figure 2. Raman spectra of 1:1 mixture of 0.0200 M NADH in bicarbonate solution, pH 9.4, and 0.0110 M ferric perchlorate in 0.1 M glycine buffer, pH 2.0: A, continuous fast flow (~3 mL/min); B, 15 min after mixing; C, 25 min; D, 40 min; E, 50 min. Experimental conditions: 514.5-nm Ar^+ excitation; laser power, 200 mW; sensitivity, 1000 counts/s; spectral slit width, 10 cm^{-1}; time constant, 2 s; scan speed, 1 cm^{-1}/s.

Major Raman bands of adenosine[7,8] were observed at 1485, 1425, 1383, 1336, 1308, and 726 cm^{-1} in neutral and alkaline solutions. Counterparts occur at similar positions for adenine and AMP. In NADH, Raman bands at 1455 (w), 1417 (s), 1374 (mw), 1337 (m), 1307 (mw), and 730 (m) most probably originate from adenosine group vibrations. None of these bands, except the ones at 1417 and 1337 cm^{-1} which lie near strong glycinate Raman bands at 1414 and 1331 cm^{-1}, were observed in the spectrum of the Fe^{III}NADH complex.

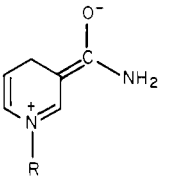
The fast-flow and time-dependent Raman spectra between 1400 and 1800 cm^{-1} of a 1:1 mixture of NADH–Fe(III) solutions containing a 2:1 ligand–metal stoichiometry are shown in Figure 2. Excitation from the 514.5-nm Ar^+ laser line occurs into the 540-nm absorption band of the blue transient species.[4,5] The major intense band in spectrum A, Figure 2, is located at 1520 cm^{-1} while a medium-weak shoulder is found at 1490 cm^{-1}. Other features include weak bands at 1690 and 1610 cm^{-1}, assignable to amide I and amide II modes, respectively. The striking feature in the spectra taken after 15 min is the appearance of an intense broad band centered at 1625 cm^{-1} and the steadily decreasing intensity of the 1520 and 1490 cm^{-1} bands. Considering the low intensity of adenine, amide, and glycinate Raman bands in spectrum A, we conclude that the 1520- and possibly the 1490-cm^{-1} bands are resonance enhanced from excitation into an allowed π(ligand) → d(metal) charge-transfer band or a ligand $\pi \rightarrow \pi^*$ transition, at 540 nm in the blue transient. The strong 1625-cm^{-1} Raman band in aged mixtures may arise either from the glycinate $\nu_a(CCO^-)$ mode or, more likely, from an amide I stretching mode of the oxidized NAD^+ species. Raman spectra of NAD^+ solutions show an intense band at 1630 cm^{-1} as the highest frequency band in the 700–1800-cm^{-1} range.[19]

Excitation at 406.7 nm in the near-ultraviolet band at 375 nm of the blue ferric NADH transient provided a Raman spectrum shown in Figure 3. A very intense band at 1690 cm^{-1} (amide I mode) and a medium band at 1490 cm^{-1} were observed in the 1400–1800-cm^{-1} region. Both of the latter bands were weakly observed with 514.5-nm excitation in spectrum B, Figure 1;

(10) Kosower, E. M. *Prog. Phys. Org. Chem.* **1965**, *3*, 81.
(11) Forrest, G. *J. Phys. Chem.* **1976**, *80*, 1127.
(12) Patrick, D. M.; Wilson, J. E.; Leroi, G. E. *J. Phys. Chem.* **1974**, *13*, 2813.
(13) Butler, I. S.; Barna, G. G. *J. Raman Spectrosc.* **1973**, *1*, 141.
(14) Adams, D. M.; Fernando, W. S. *Inorg. Chim. Acta* **1973**, *7*, 277.
(15) Hendra, P. J.; Powell, D. B. *Spectrochim. Acta* **1961**, *17*, 909.
(16) Davidson, G. *Inorg. Chim. Acta* **1969**, *3*, 596.
(17) Davidson, G.; Duce, D. A. *J. Organomet. Chem.* **1972**, *44*, 365.
(18) Bailey, R. T.; Lippincott, E. R.; Steele, D. *J. Am. Chem. Soc.* **1965**, *87*, 5346.
(19) Barrett, T. W. *J. Raman Spectrosc.* **1980**, *9*, 130.

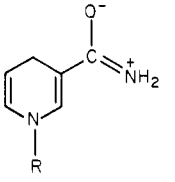
Figure 3. Raman spectrum 1:1 mixture, as given in A, Figure 2, in acidic solution. Experimental conditions: 406.7-nm Kr^+ excitation; laser power, 100 mW; sensitivity, 1 μA; spectral slit width, 8 cm^{-1}; time constant, 4.4 s; scan speed, 0.75 cm^{-1}/s.

however, the intense band at 1521-cm^{-1} that was detected with 514.5-nm irradiation was not present. This suggests that the two excited states that are associated with absorption band maxima at 375 and 540 nm differ in the region of the nicotinamide group from which they originate and/or in the symmetry of their respective states, hence, giving rise to electronic transitions with different polarizations. Differences in vibronic coupling would, therefore, be evident as differences in the relative intensities of resonance Raman bands with excitation into the 375- and 540-nm electonic absorption bands. The presence of amide I and nicotinamide ring stretching Raman bands, in resonance with the 375-nm electronic transition of the blue complex, shows that extensive electronic delocalization of amide and nicotinamide ring charge exists as previously indicated.[11,12] For both amide I at 1690 cm^{-1} and $\nu(C\cdots C) + \nu(C\cdots N)$ at 1490 cm^{-1} to be resonance intensified, the electronic excited state should involve greater perturbation of bond distances and force constants represented in the resonance form



relative to the ground electronic state parameters.

Work with other carbonyl compounds has shown that a carbonyl stretching Raman band is in resonance with an electronic absorption band, if this is due to a $\pi \rightarrow \pi^*$ transition of a conjugated double-bond system and if the C=O bond in question is involved in that conjugated double-bond system.[20] Excitation into the 340-nm absorption band of NADH was found to cause enhancement of the amide I mode at 1690 cm^{-1}, suggesting that the carbonyl group is conjugated to the ring. However, in contrast to excitation into the 375-nm band of the blue Fe^{III}NADH complex, the electronic excited state of NADH should therefore involve a large perturbation of bond distances and force constants represented by the resonance form



relative to the ground-state parameters.

Acknowledgment. This work was supported by part of an NIH Biomedical Research Support Grant (No. RR07105-04) to Clarkson College. We thank Dr. Paul Carey of the National Research Council of Canada for use of the Raman spectrometers.

(20) Nishimura, Y.; Tsuboi, M. *Science (Washington, D.C.)* **1980**, *210*, 1358.

State of Manganese in the Photosynthetic Apparatus. 1. Extended X-ray Absorption Fine Structure Studies on Chloroplasts and Di-μ-oxo-Bridged Dimanganese Model Compounds

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Contribution from the Laboratory of Chemical Biodynamics, Lawrence Berkeley Laboratory, University of California, Berkeley, California 94720. *Received December 1, 1980*

Abstract: Extended X-ray absorption fine structure studies on the manganese contained in spinach chloroplasts and on certain di-μ-oxo-bridged manganese dimers of the form $(X_2Mn)O_2(MnX_2)$ (X = 2,2′-bipyridine and 1,10-phenanthroline) are reported. From these studies, the manganese associated with photosynthetic oxygen evolution is suggested to occur as a bridged transition-metal dimer with most likely another manganese. Extensive details on the analysis are included.

Since the original observation in 1937 by Pirson[1] of an absolute requirement in photosynthesis for the trace element manganese, the specific role that Mn plays has been a subject of extensive research. The general consensus is that the Mn is directly involved in photosynthetic oxygen evolution either at the active site or as a critical component of the electron-transport chain (see review by Radmer and Cheniae[2] for further details). Until now all

(1) Pirson, A. *Z. Bot.* **1937**, *31*, 193–267.

(2) Radmer, R.; Cheniae, G. In "Topics in Photosynthesis", Vol. 2; Barber, J., Ed.; Elsevier: Amsterdam, 1977; pp 303–348.

0002-7863/81/1503-5529$01.25/0