Table I. Low-Lying Excited States of Cr^+ [a]

configuration	state	energy[b] eV	energy[b] kcal/mol
$3d^5$	a ^6S	0	0
$3d^44s$	a ^6D	1.483	34.20
$3d^44s$	a ^4D	2.421	55.84
$3d^5$	a ^4G	2.543	58.65
$3d^5$	a ^4P	2.706	62.40
$3d^5$	b ^4D	3.104	71.58
$3d^44s$	b ^4P	3.714	85.64
$3d^5$	a ^2I	3.738	86.19

[a] Data from: Sugar, J.; Corliss, C. *J. Phys. Chem. Ref. Data* 1977, *6*, 317. [b] Listed numbers are for the lowest energy *J* level of the term.

reaction appears to be exothermic (Figure 1), it must involve an electronically excited state of Cr^+.[7]

Two experiments confirm this hypothesis. The appearance potential of $CrCH_2^+$ is 2.5 ± 0.3 eV (58 kcal/mol) above the threshold for Cr^+ formation[8] from $Cr(CO)_6$. In addition, in the endothermic reaction of Cr^+ with H_2 to form CrH^+ (process 3), the threshold is shifted 2.4 eV to lower energy when Cr^+ is formed by electron impact (Figure 2).[9]

$$Cr^+ + H_2 \rightarrow CrH^+ + H \quad (3)$$

The energies of low-lying excited states of Cr^+ are summarized in Table I. An excitation energy of 2.5 eV implicates one of the lowest quartet states (the energy resolution does not distinguish among the lowest ^4D, ^4G, or ^4P states). Since the spin multiplicity of these states differs from the ground state, they should be metastable and have relatively long lifetimes.

Reaction of excited Cr^+ to form $CrCH_2^+$ is efficient and may occur on every collision. The apparent cross section is ~13 Å^2 at low energies; the actual cross section is higher because only a fraction of the Cr^+ beam is in the reactive excited state.[10] It is of interest to note that the reaction with CH_4 is promoted by *electronic* and not *translational* excitation. As the relative kinetic energy is increased, it is expected that the reaction dynamics will become dominated by direct processes.[11] It is thus not surprising that ground-state Cr^+ reacts with CH_4 to form only CrH^+, probably in a stripping reaction.[12] Formation of $CrCH_2^+$ requires major rearrangement and a relatively long-lived intermediate. Hence electronic excitation is uniquely effective in promoting this reaction.

Transition-metal carbenes are believed to be intermediates in processes such as hydroformylation and the Fischer–Tropsch synthesis. Our results show that excited chromium ions produce chromium carbene ions exothermically from methane. This suggests that photochemical methods may provide an efficient way to form these highly reactive intermediates. For example, photoexcitation of metal atoms in a matrix containing methane might lead to carbene formation.[13]

We have observed numerous other examples where electronically excited metal ions exhibit modified reactivity in ICR and ion beam studies of organometallic chemistry.[14] This clearly indicates that caution be exercised in the inference of thermochemical data from the observation of reactions which *appear* to be exothermic.[15]

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(7) In a note added in proof, Freas and Ridge note that studies of the kinetics of the reaction of Cr^+ with $Cr(CO)_6$ examined by use of an ion cyclotron resonance spectrometer suggest two states of Cr^+ are produced by electron impact from $Cr(CO)_6$ (Freas, R. B.; Ridge, D. P. *J. Am. Chem. Soc.* **1980**, *102*, 7129).

(8) Appearance potential curves were taken on an ion cyclotron resonance spectrometer operating in the drift mode. The shift in threshold is calculated by using the extrapolated voltage difference method as described in: Warren, J. W. *Nature* (London) **1950**, *165*, 810. Variation of electron energy in the ion beam experiments produces the expected variation in cross sections; however, the intensity of the beam near threshold is too low to obtain accurate data. Earlier appearance potential measurements of Cr^+ produced by electron impact from $Cr(CO)_6$ suggested that chromium ions may be produced in an excited state. See: Winters, R. E.; Kiser, R. W. *Inorg. Chem.* **1965**, *4*, 157. However, subsequent measurements have not agreed well with this result nor with each other: Rosenstock, H. M.; et al., *J. Phys. Chem. Ref. Data Suppl.* **1977**, *6*, No. 1. If the appearance potential curve of Cr^+ that we have observed is due to an excited state, then the difference of 2.5 eV corresponds to a lower limit on the energy of the reactive excited state.

(9) Armentrout, P. B.; Beauchamp, J. L. *Chem. Phys.* **1980**, *48*, 315.

(10) The apparent cross section for reaction of excited Cr^+ with H_2 (process 3) is about 8 times the maximum cross section of ground-state Cr^+ (Figure 2), indicating that here, too, the excited state reaction is efficient.

(11) Levine, R. D.; Bernstein, R. B. "Molecular Reaction Dynamics"; Oxford University Press: New York, 1974.

(12) Henglein, A. *Adv. Chem. Ser.* **1966**, *No. 58*, 63.

(13) A recent study (Billups, W. E.; Konarski, M. M.; Hauge, R. H.; Margrave, R. H. *J. Am. Chem. Soc.* **1980**, *102*, 7394) indicates that photoexcitation of metal atoms in a methane matrix yields the insertion product CH_3MH where M = *Mn, Fe, Co, Cu, Zn*, Ag, and Au. Ca, Ti, Cr, and Ni do not react. Further irradiation yields $(CH_3)_2M$. Although they might be difficult to characterize, no mention was made of carbenes in the systems studied. It appears that in the frozen matrix the first intermediate suggested in Scheme I is trapped. Further excitation results in reaction with a second methane molecule rather than an α-hydrogen shift which would yield a carbene. In a dilute system (e.g., methane in argon) it might be possible to observe the carbene.

(14) See, for example: Foster, M. S.; Beauchamp, J. L. *J. Am. Chem. Soc.* **1975**, *97*, 4808. We have observed reactions of excited-state Cr^+ as well as excited-state Mn^+ [produced by electron impact of $Mn_2(CO)_{10}$] with alkanes other than methane which differ markedly from the reactions of ground-state ions.

(15) For examples where this analysis has been used, see: Allison, J.; Freas, R. B.; Ridge, D. P. *J. Am. Chem. Soc.* **1979**, *101*, 1332. Allison, J.; Ridge, D. P. *Ibid.* **1979**, *101*, 4998 and ref 4a. In the latter study, thresholds for $MnCH_2^+$ product ions were checked and found to be identical with the threshold for Mn^+ formation.

First Evidence for Manganese Binding to Sulfur Donor Group in Metalloprotein, Mn(III)-Containing Acid Phosphatase

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Although the biological importance of iron–sulfur complex systems and the chemical similarity of Fe(III) and Mn(III) are well-known, manganese binding to sulfur donor groups in metalloproteins has never been demonstrated. In addition, the chemistry of manganese complexes with sulfur donor ligands is much less understood than that for oxygen and nitrogen donor ligand complexes.[1] The acid phosphatase purified from sweet potato contains one Mn(III) ion per molecule which is essential for enzymatic activity and the intense 515-nm visible band.[2,3] We reported the coordination of a tyrosine phenolate anion to the Mn(III) chromophore of this enzyme.[2] However, a Raman line due to the Mn–S streching mode was not detected because of the fluorescence of the native enzyme. The present study of the tryptophan-modified Mn(III)-containing acid phosphatase has demonstrated the first evidence for manganese binding to sulfur

(1) Lawrence, G. D.; Sawyer, D. T. *Coord. Chem. Rev.* **1978**, *27*, 173–193. This is due mainly to the facile oxidation of sulfur in such ligands.

(2) Sugiura, Y.; Kawabe, H.; Tanaka, H. *J. Am. Chem. Soc.* **1980**, *102*, 6581–6582.

(3) Recently, the iron-containing acid phosphatase from pig allantoic fluid was purified to homogeneity and the violet enzyme showed an absorption maximum near 550 nm (ϵ 2000): Keough, D. T.; Dionysius, D. A.; Jersey, J.; Zerner, B. *Biochem. Biophys. Res. Commun.* **1980**, *94*, 600–605.

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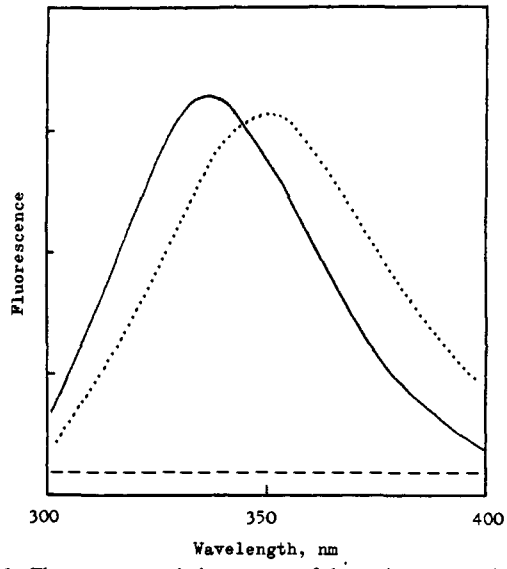

Figure 1. Fluorescence emission spectra of the native enzyme (—), 6 M guanidine treated enzyme (---), and NBS treated enzyme (– – –) in acetate buffer (pH 5.8). The protein concentration was 5×10^{-6} M, and the excitation was at 280 nm.

donor group in metalloproteins.

Maximal fluorescence intensity for the native Mn(III)-containing enzyme occurred at 335 nm, while that for the 6 M guanidine hydrochloride treated enzyme was observed at 350 nm (see Figure 1). Even if the excitation wavelength is varied from 270 to 295 nm, no evidence for significant tyrosine energy transfer was detected. In adrenal iron–sulfur protein, an anomalous emission (331 nm) of the tyrosyl residue at position 82 of the native protein has been observed.[4] However, the apoprotein exhibited a normal tyrosine emission at 304 nm. Thus, the fluorescence in this Mn(III) enzyme is dominated by its tryptophan residues. The 15-nm blue shift from the denaturated enzyme and standard *N*-acetyltryptophan methyl ester strongly indicates that several of the 24 tryptophans of the native enzyme[5] must be within considerably hydrophobic environment. The Mn(III)-containing acid phosphatase activity was significantly abolished by the tryptophan modification with *N*-bromosuccinimide (NBS).[6] However, the Mn(III) optical activity at the characteristic 515-nm band was unaffected by the NBS modification. Oxidation of the tryptophans by NBS also led to a disproportionately large decrease in the fluorescence intensity.[7] Therefore, the effect of the NBS modification on the tryptophane fluorescence appears to reflect environmental and conformational changes in the active site locus rather than direct quenching or intersystem crossing effects due to contact interaction between the Mn atom and tryptophans.[8] Indeed, it is difficult to imagine how a tryptophan indole can be an endogenous ligand to the Mn(III). A similar profound effect of tryptophan residues on the catalytic activity and fluorescence has been demonstrated in galactose oxidase which contains 1 Cu(II) ion and 18 tryptophans per molecule.[9]

Figure 2 compares resonance Raman spectra for the NBS-treated enzyme (A) and native enzyme (B) obtained with 5145-Å

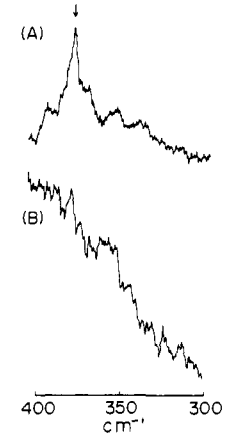

Figure 2. Resonance Raman spectra of the tryptophan-modified enzyme (A) and native enzyme (B). The sample concentration was 0.5 mM, and the spectra were measured at pH 5.8 and 20 °C (A) and pH 6.8 and 4 °C (B). Instrumental conditions were as follows: excitation, 514.5-nm line of Ar^+ laser; power, 40 (A) and 20 mW (B) at a sample point; time constant, 8 (A) and 16 s (B); slit width, 250 (A) and 200 μm (B); scan speed, 10 cm^{-1}/min.

excitation. The tryptophan-modified enzyme showed a positive band at 370 cm^{-1}, though the Raman bands of the native enzyme between 300 and 400 cm^{-1} are obscured by the fluorescent background.[10] The typical Raman line at 370 cm^{-1} is preferentially assigned to a Mn(III)–S streching mode.[11] Such Mn(III)–S modes at ca. 370 cm^{-1} have been recorded in the IR spectrum of tris(*N*,*N*-diethyldithiocarbamato)manganese(III) complex.[12] In addition, symmetric streching vibrations of sulfhydryl sulfur to Fe(III) bonds have been assigned at 315–365 cm^{-1} in iron–sulfur proteins and synthetic iron–sulfur clusters.[13] The argument for Mn(III)–S(cysteine) streching mode is strengthened by chemical evidence for the presence of cysteine at the Mn(III) active site. The sulfhydryl determination by the Ellman method revealed tht free sulfhydryl groups were not detected in the native Mn(III) enzyme but 1 mol of SH group/enzyme was determined in the 6 M guanidine treated denaturated enzyme.[14] The binding of *p*-chloromercuribenzoate, mercury(II), or lead(II) ion to the Mn(III)-containing native enzyme strongly inhibited the phosphatase activity and was concomitant with the loss of the violet color. These results indicate that the native Mn(III)–enzyme has at least one cysteine residue per Mn(III) ion, available for Mn(III)–S coordination. With respect to the active-site donor sets, the Mn(III)-containing acid phosphatase with tyrosine oxygen[2] and cysteine sulfur donors is distinctly different from the Zn(II)-containing alkaline phosphatase which consists of at least three histidyl nitrogen donors.[15]

In conclusion, the present paper has provided the first evidence for manganese–sulfur binding in metalloprotein, Mn(III)-containing acid phosphatase.

Acknowledgment. We are grateful to Dr. T. Kitagawa for the resonance Raman measurements. This study was supported in part by a grant from the Ministry of Education, Science, and Culture, Japan.

(4) (a) Kimura, T.; Ting, J. *Biochem. Biophys. Res. Commun.* **1971**, *45*, 1227–1231. (b) Kimura, T.; Ting, J.; Huang, J. J. *J. Biol. Chem.* **1972**, *247*, 4476–4479.

(5) The amino acid composition of the Mn(III)-containing acid phosphatase showed that the enzyme contains 24 tryptophan residues per molecule.

(6) The tryptophan-modified enzyme was prepared by the treatment of NBS (100-fold molar quantity per enzyme) to the native enzyme at pH 5.8.

(7) Imoto, T.; Forster, L. S.; Rupley, J. A.; Tanaka, F. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 1151–1155. Oxindole groups were assumed to make no significant fluorescence contribution.

(8) The CD spectra suggest that the NBS-modified enzyme has somewhat different conformation of the active site locus from that for the native enzyme.

(9) (a) Kosman, D. J.; Ettinger, M. J.; Bereman, R. D.; Giordano, R. S. *Biochemistry* **1977**, *16*, 1597–1601. (b) Weiner, R. E.; Ettinger, M. J.; Kosman, D. J. *Ibid.* *16*, 1602–1606.

(10) It is not clear whether the improvement in the Raman spectrum upon modification is simply due to tryptophan quenching alone.

(11) Other metal–ligand streching modes, for example Mn–O(phenolate) mode, could contribute in this region and have not been excluded conclusively.

(12) Healy, P. C.; White, A. H. *J. Chem. Soc. Dalton Trans.* **1972**, 1883–1887.

(13) (a) Long, T. V.; Loeher, T. M.; Alkins, J. R.; Lovenberg, W. J. *J. Am. Chem. Soc.* **1971**, *93*, 1809–1811. (b) Tang, S. P. W.; Spiro, T. G.; Autanaitis, C.; Moss, T. H.; Holm, R. H.; Herskovitz, T.; Mortenson, L. E. *Biochem. Biophys. Res. Commun.* **1975**, *62*, 1–6. (c) Holm, R. H.; Ibers, J. A. "Iron-Sulfur Proteins"; Lovenberg, W., Ed.; Academic Press: New York, 1977; pp 205–281, Vol. III.

(14) The amino acid analysis showed that the Mn(III) enzyme has three half-cystine [(1) three cysteine or (2) one cysteine and one cystine] residues.

(15) Coleman, J. E.; Chlebowski, J. F.; Otvos, J. D.; Schoot Uiterkamp, A. J. M.; Armitage, I. M. *Trans. Am. Crystallogr. Assoc.* **1978**, *14*, 17–57.