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Bond Valence Sum Analysis of Metal–Ligand Bond Lengths in Metalloenzymes and Model Complexes

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Received August 30, 1991

Bond valence sums (BVS's) have been calculated for a series of model complexes for important metalloenzymes. Values calculated from crystallographic distances predict the correct oxidation state of the metal center in a wide range of structural types. Many of the selected complexes have previously been studied by X-ray absorption spectroscopy, and first-shell metal–ligand bond distances have been determined by analysis of extended X-ray absorption fine structure (EXAFS). BVS's determined from the EXAFS distances also predict the correct metal oxidation states. Extension of the method to actual metalloproteins shows that the correct oxidation state can also be calculated from EXAFS distances in natural systems. The method can be applied to structures as widely varied as blue copper centers, oxo-bridged diiron centers, and mixed-valence forms of Fe–S clusters. The approach is then used to test possible oxidation-state assignments for the manganese clusters in the S_1 and S_2 states of the oxygen-evolving complex in photosystem II.

The elucidation of the active-site structure of metalloenzymes is of prime importance in understanding the mechanisms of many important biochemical reactions, and X-ray absorption spectroscopy has played a key role in this regard. Metal–ligand bond distances can be obtained from analysis of extended X-ray absorption fine structure (EXAFS),[1-3] and when protein crystallographic data have become available, the EXAFS distances have generally been in good agreement with the crystallographic distances.[3-5] In addition, information regarding the oxidation state of the metal ion or ions is also available from analysis of X-ray absorption near-edge spectroscopy (XANES).[1,6] Correlations of edge features with ligand hardness, coordination charge, metal–ligand bond distances, and coordination numbers has been observed and can be interpreted in terms of the relevant models of oxidation state and structure.[6-8]

An empirical quantity, known as the bond valence sum (BVS), has been used to determine the oxidation state of metal ions in solids based on crystallographically determined metal–ligand bond distances.[9] In analyses of high-temperature superconductors, BVS's have been effective for assessing important factors such as oxidation state and the role of steric effects in determining T_c.[10,11] The method has also been applied to crystallography of coordination complexes to verify the assignment of metal ion oxidation states.[12]

Bond valences (s) are calculated according to eq 1, where r is the observed bond distance and r_0 and B are empirically determined parameters.[9] The values of r_0 for many metal–ligand

$$s = \exp[(r_0 - r)/B] \tag{1}$$

interactions are tabulated in ref 9, and the remaining values can be calculated using a simple formula:

$$r_0 = r_c + A(r_a) + P - D - F \tag{2}$$

Here A is 0.8 for transition-metal ions and 1 for other ions and r_c and r_a are specific values for the cation and anion in the bond and are tabulated in ref 9. The quantities D, P, and F are corrections for the number of p, d, and f electrons in the cation. Values of D are tabulated in ref 9, and P and F are determined by

$$P = 0.0175(\text{cation period} - 2) \tag{3}$$

$$F = 0.016(\text{number of f electrons}) \tag{4}$$

Table I. Values of r_0 Used To Calculate Bond Valence Sums

bond	r_0, Å	bond	r_0, Å
Fe^{3+}–O	1.759	Mn^{2+}–O	1.790
Fe^{3+}–N	1.855	Mn^{2+}–N	1.862
Fe^{3+}–S	2.149	Mn^{3+}–O	1.760
Fe^{2+}–O	1.734	Mn^{3+}–N	1.832
Fe^{2+}–N	1.806	Mn^{4+}–O	1.753
Fe^{2+}–S	2.111	Mn^{4+}–N	1.825
Cu^{2+}–N	1.751	Mn^{5+}–O	1.773
Cu^{2+}–O	1.679	Mn^{5+}–N	1.845
Cu^{2+}–S	2.054	Zn^{2+}–O	1.776
Cu^{+}–N	1.595	Zn^{2+}–N	1.704

The BVS for a given metal center can then be determined using values for r_0 either tabulated in ref 9 or calculated according to eq 2. Bond valences for each bond are calculated from eq 1 using a value of 0.37 for B. The BVS of a given metal center can then be determined by summing the values of s for each metal–ligand bond.

In this article, I will present bond valence sums for crystallographically determined structures of biologically relevant metal complexes. These sums show that the method can be used to predict the oxidation state of metal ions in mono-, di-, tri-, and tetranuclear complexes. Many of these have also been analyzed by EXAFS, and the BVS's calculated from the EXAFS distances show satisfactory agreement with those from crystallography. The method can also be applied to metalloproteins exhibiting mono-, di-, and tetranuclear metal sites whose structures have been determined by EXAFS (and crystallography where available). In the metalloprotein systems, the BVS method predicts the correct oxidation state in systems where structures are known. This suggests the interesting possibility that BVS's calculated from first-shell EXAFS distances can be used to test models for metalloenzyme active sites whose structures or oxidation states are not known unambiguously. The application of this idea to the oxidation state of the manganese cluster in photosystem II provides additional information that might prove useful in continuing efforts to elucidate the structure of this important metal cluster.

Model Complexes

Examples from the literature of a wide range of crystallographically characterized metal complexes that are relevant to important metalloenzymes were collected.[13-30] The important

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Table II. Bond Valence Sums for Metalloenzyme Model Complexes Calculated from EXAFS and X-ray Crystallography[a]

complex	BVS (EXAFS)	BVS (X-ray)	ox. state	ref
$Cu(HIm)_4^{2+}$	1.98	1.99	2.0	13
$Zn(HIm)_4^{2+}$	2.12	2.18	2.0	14
$Fe(acac)_3$	3.14	3.20	3.0	4, 15
$(Me_3tacn)_2Fe_2(OH)(OAc)_2^+$	1.77	2.01	2.0	4, 16
$Fe_2(H_2Hbab)_2(NMeIm)_2$		2.07	2.0	17
$Fe_2(BPMP)(OPr)_2^+$		2.16	2.0	18
$Fe_2(BPMP)(OPr)_2^{2+}$		2.68	2.5	18
$(tacn)_2Fe_2O(OAc)_2^{2+}$	3.05	3.15	3.0	4, 19
$Fe_2(TPA)_2O(OAc)^{3+}$		3.19	3.0	20
$[HB(pz)_3Fe]_2O(OAc)_2$	3.16	3.16	3.0	4, 21
$Fe_4O_2(OH)_4(tacn)_4^{4+}$	3.07	2.99	3.0	23
$Fe_2O(O_2CH)_4(BIPhMe)_2$	2.97	3.18	3.0	32
$Mn_4O_2(O_2CCPh_3)_6(OEt_2)_2$		2.68	2.5	24
$Mn_4O_2(O_2CCH_3)_6(bpy)_2$		2.60	2.5	25
$Mn_4O_2(tphpn)_2(H_2O)_2(CF_3SO_3)_2^{3+}$		2.89	2.75	26
$Mn_4O_2(O_2CCH_3)_7(bpy)_2^+$		3.21	3.0	25
$Mn_4O_3Cl_6(HIm)(OAc)_3^{2-}$		3.21	3.25	27
$[(Mn_2O_2)_2(tphpn)_2]^{4+}$		3.63	3.5	28
$Mn_3O_4(OH_2)_2(bpy)_4^{4+}$		4.09	4.0	29
$Mn_4O_6(tacn)_4^{4+}$		4.22	4.0	30

[a] Abbreviations: HIm = imidazole, acac = acetonylacetonate, Me_3tacn = 1,4,7-trimethyl-1,4,7-triazacyclononane, tacn = 1,4,7-triazacyclononane, bpy = 2,2′-bipyridine, Htphpn = *N,N,N′,N′*-tetrakis(2-pyridylmethyl)-2-hydroxypropane-1,3-diamine, H_4Hbab = 1,2-bis(2-hydroxybenzamido)-benzene, HBPMP = 2,6-bis[bis(2-pyridylmethyl)pyridylmethyl)amine, BIPhMe = bis(1-methylimidazolyl)phenylmethoxymethane.

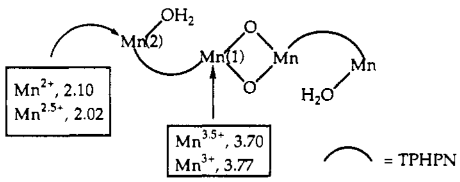

Figure 1. Schematic representation of the structure of $Mn_4O_2(TPHPN)_2(H_2O)_2(CF_3SO_3)_2^-$. The calculated values for the bond valence sums are shown for models where Mn(2) = Mn^{2+} and Mn(1) = $Mn^{3.5+}$ or Mn(2) = $Mn^{2.5+}$ and Mn(1) = Mn^{3+}; see text.

Table III. Bond Valence Sums for Metalloprotein Active Sites Calculated from EXAFS Metal–Ligand Bond Distances

protein	coordination	BVS	ox. state	ref
plastocyanin	2-N, 2-S	1.95	2.0	5
Cu PPAO	1-S, 2-N, 2-O	2.05	2.0	31
red Cu SOD	3-N	1.18	1.0	13
Cu-Zn SOD (Cu)	4-N	2.04	2.0	14
Cu-Zn SOD (Zn)		2.00	2.0	14
$semimetHrN_3$	3-O, 3-N	2.59	2.5	4
reduced-MMO	3-O, 2.5-N	1.98	2.0	32
semimet-MMO	3-O, 2.5-N	2.49	2.5	32
oxidized-MMO	3-O, 2.5-N	2.85	3.0	32
$metHrN_3$	3-O, 3-N	3.07	3.0	4
$Fe_4S_4^+$	4-S	2.33	2.25	33
$Fe_4S_4^{2+}$	4-S	2.60	2.5	33

r_0 values are set out in Table I and can be used to calculated the BVS's by summing the bond valences calculated from eq 1. For example, the complex $Fe_2(BPMP)(OPr)_2^+$ exhibits two six-coordinate Fe(II) centers. One center contains three Fe^{2+}–O bonds of 2.062, 2.035, and 2.150 Å and three Fe^{2+}–N bonds of 2.259, 2.232, and 2.165 Å. The bond valence of each bond is calculated using eq 1. Since the r_0 for Fe^{2+}–O is 1.734 Å (Table I), the 2.062-Å bond gives a bond valence of $\exp[(1.734-2.062)/0.37] = 0.412$. The bond valences of the remaining two bonds are calculated similarly and added to the bond valences of the three Fe^{2+}–N (r_0 = 1.806 Å) linkages to give a BVS of 2.17 for the Fe(II) center. The other Fe(II) center exhibits three Fe^{2+}–O bonds of 2.052, 2.045, and 2.138 Å and three Fe^{2+}–N bonds of 2.250, 2.241, and 2.178 Å. The bond valences are calculated for each of these bonds as above and summed to give a BVS of 2.15 for the other Fe(II) center. The average BVS for the dinuclear complex is then the average of the BVS's for each Fe(II) center of 2.16, which is close to the known average oxidation state of 2.0. The average BVS's for a series of model complexes have been calculated similarly and are set out in Table II.

Clearly, the method predicts the correct oxidation states for monomers such as $Cu(HIm)_4^{2+}$, $Zn(HIm)_4^{2+}$, and $Fe(acac)_3$. The method also predicts the correct oxidation states for metal centers in complex structures such as oxo-bridged dinuclear Fe complexes and tri- and tetranuclear complexes of high-valent Mn. From inspection of the diiron examples, it appears that the error is <0.25 units. Since in the dinuclear cases the oxidation states can only differ by 0.5 units, this level of error is acceptable for differentiating between 2.0, 2.5, and 3.0 oxidation states. Importantly, the correct oxidation state is predicted for $Fe_2(H_2Hbab)_2(NMeIm)_2$ which exhibits two five-coordinate centers,[17] suggesting that the method accurately accounts for different coordination numbers.

In the tetranuclear clusters, where average oxidation levels differing by only 0.25 are possible, application of the BVS alone is not sufficient to determine the average oxidation state. EPR spectroscopy, however, can be used to determine whether the complex is in a Kramer's state and, consequently whether the cluster contains an even or odd number of valence electrons.[27,29] Thus, the BVS will only be required to differentiate between oxidation levels that differ by 0.5, which is possible given an error of the BVS of <0.25. For example, the $Mn_4O_6(tacn)_4^{4+}$ cluster gives a BVS of 4.22, which might be compatible either with an oxidation level of 4.0 or 4.25. The complex is known to be EPR silent,[30] however, ruling out the 4.25 oxidation level. Thus, the BVS supports an oxidation level of 4.0, which is the known oxidation state for this complex. This consideration will be important in interpreting the BVS of photosystem II.

The cluster $[Mn_4O_2(TPHPN)_2(H_2O)_2(CF_3SO_3)_2]^{3+}$,[26] whose structure is shown schematically in Figure 1, presents an interesting opportunity to apply the BVS method to a model complex. In the crystallographically determined structure, there is an inversion center that results in the presence of only two unique Mn ions, even though the average Mn oxidation state is 2.75. The possible assignments are therefore (II, III, IV, II) or (II, III, III, III), i.e., Mn(2) is Mn^{2+} and Mn(1) results from a crystallographically disordered (III, IV) pair, or Mn(1) is Mn^{3+} and Mn(2) results from a disordered (II, III) pair.

The BVS's can be calculated for each of these assignments from the crystallographically determined distances (Figure 1). For the (II, III, IV, II) assignment, Mn(2) is modeled as an Mn^{2+} ion and has a BVS of 2.10 and Mn(1) is modeled as $Mn^{3.5+}$ and gives

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a BVS of 3.70. For the +3.5 oxidation state, the BVS is the average of the values determined from placing both Mn^{3+} and Mn^{4+} ions in the Mn(1) site. Similarly, the (II, III, III, III) assignment gives BVS's of 2.02 for an $Mn^{2.5+}$ ion in the Mn(2) site and 3.77 for an Mn^{3+} ion in the Mn(1) site. The (II, III, IV, II) assignment clearly gives BVS values in better agreement with the appropriate Mn oxidation states. This calculation supports the same assignment made on the basis of other physical properties.[26]

In some cases shown in Table II, distances are available from both crystallography and EXAFS.[4,13-16,19,21,22] The BVS's calculated from the EXAFS distances also give the expected oxidation states and agree well with those calculated from the crystallographic distances. The largest difference between BVS's determined from both crystallographic and EXAFS distances is 0.24 for $(Me_3tacn)_2Fe_2(OH)(OAc)_2^+$, which is within the previously stated resolution of 0.25 units. Importantly, there is acceptable agreement even in complex molecules such as $(tacn)_2Fe_2O(OAc)_2^{2+}$, $Fe_2O(O_2CH)_4(BIPhMe)_2$, and $Fe_4O_2(OH)_4(tacn)_4^{4+}$, where the EXAFS analyses give one short distance for the O^{2-} bridges and an average long distance for the other ligands.[4,23]

Metalloproteins

The BVS values calculated from EXAFS distances for a wide range of metalloprotein active sites are shown in Table III.[4,5,13,14,31-33] The method gives values within ±0.18 of the expected oxidation states for mononuclear sites found in plastocyanin, pig plasma amine oxidaste (PPAO), and reduced Cu superoxide dimutase (SOD); dinuclear sites found in met- and semimet-hemerythrin azide (met- and semimetHrN_3), methane monooxygenase (MMO), and Cu–Zn SOD; and tetranuclear sites in the $Fe_4S_4^+$ and $Fe_4S_4^{2+}$ forms of the nitrogenase iron protein.

To calculate the values shown in Table III, structural models had to be assumed. For plastocyanin,[34] metHrN_3,[35] and Cu–Zn SOD,[36] the structures are known from crystallography. For the reduced Cu SOD site, a three-coordinate Cu(I) with all-nitrogen coordination was used.[13] For semimetHrN_3, the structure was assumed to be analogous to metHrN_3, with each iron exhibiting one short Fe–O, two long Fe–O, and three Fe–N bonds.[4] For these oxo-bridged iron sites, a short Fe–O bond distance and a long Fe–ligand distance are obtained from the EXAFS analyses. The BVS was calculated by using the short distance for the oxo bridge and the long distance for all of the other ligands. For semimetHrN_3, the reported value is the average of values calculated for Fe^{2+} and Fe^{3+}. Four-coordinate Fe sites with all-sulfur ligation were assumed for the two forms of the $Fe_4S_4^{n+}$ protein.[33]

The agreement for all three redox forms of methane monooxygenase is particularly encouraging. The coordination model assumed was three oxygen ligands and 2.5 nitrogen ligands for Fe^{II}_2, $Fe^{II}Fe^{III}$, and Fe^{III}_2 complexes in the reduced, semimet, and oxidized forms, respectively. In the semimet form, the Fe(III) was calculated as six-coordinate, with three nitrogen and three oxygen ligands, and the Fe(II) was calculated as five-coordinate, with two nitrogen and three oxygen ligands. Reversal of this assumption to a five-coordinate Fe(III) and six-coordinate Fe(II) model does not alter the BVS dramatically. From the BVS values, it is clear that the method accounts for the changes in oxidation state of the three forms quite well. In addition, the method accounts well for the presence of short metal–μ-O^{2-} bonds. This is evident from the reasonable values determined for the diiron enzymes both with (Hr) and without (MMO) a short Fe–O bridge.[4,32]

Photosystem II

A variety of structures have been proposed for the manganese cluster in photosystem II.[37-40] Recent EPR results show that a tetranuclear formulation for the S_2 state is extremely likely.[41] Pulsed EPR studies suggest that Mn coordination is largely by oxygen; there are no sulfur ligands and at most 1–2 nitrogen ligands for the entire cluster.[42] Recent EXAFS results by Klein and co-workers[43] on the S_1 state show a first shell that is fit best by a short Mn–O distance at 1.76 Å and a longer Mn–O distance at 2.25 Å. However, Penner-Hahn et al.[7] see no evidence for a short Mn–O interaction, but do observe an average Mn–O interaction of 1.91 Å, similar to the average distance observed by George et al.[44] Klein and co-workers have also reported that the S_2 state exhibits an edge shift consistent with oxidation of Mn in the $S_1 \rightarrow S_2$ transition, but little or no change in the average Mn–O bond length is observed.[43]

Application of the BVS method to determination of the oxidation state of Mn in PS II would require only the average Mn–O bond length and the average coordination number for Mn in the protein. There is some agreement on the average Mn–O bond length of 1.91 Å,[7,43,44] and most workers agree that the Mn centers are either five- or six-coordinate.

The BVS for PS II can be calculated to be 3.3 for all five-coordinate Mn or 4.0 for all six-coordinate Mn. Since the average Mn–O bond length does not appear to change between S_1 and S_2, these values apply to both states. Because the r_0 values for Mn^{3+}, Mn^{4+}, and Mn^{5+} are similar (Table I), these BVS's vary by only ±0.03 when values of r_0 are used for different Mn oxidation states. Similarly, the presence of only one or two N-ligands does not alter the calculated values significantly.

A number of different oxidation states have been proposed for the Mn clusters in PS II. From EPR and XANES results, the assignments for S_1 that seem feasible are (III)$_4$, (III)$_2$(IV)$_2$, and (IV)$_4$.[37] As discussed above, the EPR results rule out the (III)$_3$(IV) and (III)(IV)$_3$ assignments for S_1, so that the BVS is only required to differentiate between oxidation levels that differ by 0.5 rather 0.25. Since S_2 is oxidized by one electron relative to S_1, the corresponding feasible assignments for S_2 are (III)$_3$(IV), (III)(IV)$_3$, and (IV)$_3$(V), respectively. A BVS between 3.3 and 4.0 for both states would argue strongly for the (III)$_2$(IV)$_2$ assignment for S_1, since values of 3.50 for S_1 and 3.75 for S_2 both fall within the calculated range. In contrast, the predicted BVS values for the other two assignments fall outside the calculated range in either the S_1 or S_2 states. In the S_1 = (III)$_4$ assignment, a value of 3.0 in S_1 is too low, and in the S_1 = (IV)$_4$ assignment, a value of 4.25 for S_2 is too high. It is possible to calculate the average bond lengths that would give BVS's consistent with the (III)$_4$ or (IV)$_4$ assignments for S_1. These calculations show that the average bond length would have to be >0.04 Å higher or lower for the BVS to support the (III)$_4$ or (IV)$_4$ assignments, respectively. This deviation is significantly larger than the actual EXAFS experimental error of 0.01–0.02 Å.[7]

The XANES of S_1 has been noted to be consistent with the (III)$_2$(IV)$_2$ assignment.[45,46] Also, the parallel polarization EPR spectrum of a (III)$_2$(IV)$_2$ dimer-of-dimers complex prepared by

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Armstrong and Chan[28] is strikingly similar to that of the S_1 state of PS II, measured by Klein and co-workers.[47] Since the S_2-state EPR spectrum can be simulated with either a $(III)_3(IV)$ or $(III)(IV)_3$ tetramer,[37,48] the assignment supported by the BVS results is also consistent with the EPR results. It should be noted, however, that while the tetranuclear formulation is gaining increasing support and appears extremely likely to be correct,[41] there are other models which, though rather cumbersome, have not been definitively ruled out. If the tetranuclear formulation does not survive continued experimentation, the BVS results may have to be reconsidered. Nevertheless, the method should still be applicable in light of any new information that may become available in the future.

Conclusions

The bond valence sum method is a powerful tool in analyzing the compatibility of a given set of crystallographically determined bond distances with a particular metal oxidation state.[9] The calculations performed for the model complexes show that the method can be applied to crystallographically determined distances in metalloenzymes that have also been determined using EXAFS. This presents the intriguing possibility that the method may be used to gain insight into metalloprotein active sites that have been studied by EXAFS. The values in Table III suggest that the method works quite well for a variety of structures, including mixed-valence units and complex ligation environments such as those found in the oxo-bridged diiron enzymes.

When applied to the Mn cluster in PS II, the BVS method can distinguish between assignment of the tetramer to the $(III)_4$, $(II)_2(IV)_2$, or $(IV)_4$ oxidation states in S_1. The calculated BVS's are most compatible with the $(III)_2(IV)_2$ assignment and can account for the failure of the average Mn–O bond distance to decrease significantly upon oxidation of one of the Mn centers to form the S_2 state. Since EXAFS is such a powerful technique for determining bond lengths, the BVS method presents a potentially attractive complement to XANES analysis for determining oxidation states of metal centers in metalloproteins.

Acknowledgment. I thank Drs. G. W. Brudvig, C. C. Torardi, and J. E. Penner-Hahn for helpful discussions; the donors of the Petroleum Research Fund, administered by the American Chemical Society, for financial support; the Camille and Henry Dreyfus Foundation for a New Faculty Award; the National Science Foundation for a Presidential Young Investigator Award; and the David and Lucile Packard Foundation for a Fellowship in Science and Engineering.

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Variable Photon Energy Photoelectron Spectroscopy of OsO_4 and Pseudopotential Calculations of the Valence Ionization Energies of OsO_4 and RuO_4

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Received August 6, 1991

Relative partial photoionization cross sections and photoelectron branching ratios have been obtained for the valence bands of osmium tetraoxide in the ionization energy range 12–18 eV. The photon energies used ranged between 24 and 115 eV. The ionization cross sections of the $2t_2$, 1e, and $2a_1$ orbitals show evidence of substantial metal character. By use of an ab initio many-body Green's function formalism that takes into account the effect of electron correlation and relaxation, ionization energies of OsO_4 and RuO_4 have been calculated. Considerations of the spectral features and the predictions of the calculation lead to an assignment of ion state ordering of $^2T_1 < {}^2T_2 < {}^2A_1 < {}^2E < {}^2T_2$. The presence of a substantial p–d resonance feature in the cross section of the upper $^2T_2$ and ^2E ion states shows that the $2t_2$ and 1e orbitals have significant Os 5d character. The lower $^2T_2$ ion state (arising from ionization from the $3t_2$ orbital) undergoes a spin–orbit splitting of 0.4 eV resulting from an Os 6p contribution to the $3t_2$ molecular orbital.

Introduction

The volatility, high symmetry, and classic character of OsO_4 led to both this molecule and the closely related RuO_4 being early targets in the application of gas-phase photoelectron spectroscopy (PES) to the study of bonding in transition metal complexes.[1-5] RuO_4 proved a difficult candidate for investigation as both CO_2 and H_2O accompanied the spectrum in the early stages of measurement and the electron multipliers were attacked, leading to poor instrumental performance.[1-4] Once the ambiguities, due to impurities, in the number of spectral bands were resolved there was general agreement on the correlation of bands between the two compounds. Various assignments were proposed for the spectra of these two compounds, which are summarized along with the ionization energies (IEs) in Table I. Subsequently, two theoretical papers concurred[6,7] in revising the assignment of the photoelectron (PE) spectrum of OsO_4. These assignments are also given in Table I. Details of the various empirical arguments

Table I. Ionization Energies and Assignments of the PE Spectra of OsO_4 and RuO_4

	vertical IE/eV[a]		ref				
band	OsO_4	RuO_4	1[b]	2	3, 4	6	7
1	12.35	12.15	$^2T_1$	$^2T_2$	$^2T_1$	$^2T_1$	$^2T_1$
2	13.14	12.92	$^2T_2(U')$	$^2T_1$	$^2T_2$	$^2T_2$	$^2T_2$
3	13.54	13.01	$^2T_2(E'')$	$^2A_1$	^2E	$^2A_1$	$^2A_1$
4	14.66	13.93	^2E	^2E	$^2A_1$	^2E	^2E
5	16.4, 16.8	16.1	$^2T_2$	$^2T_2$	$^2T_2$	$^2T_2$	$^2T_2$

[a] IE values are taken from refs 3 and 4, where IE values for the observed vibrational fine structure are also given. [b] Diemann and Muller assigned the $^2A_1$ ion state to a band at 15.58 eV in the spectrum of OsO_4 and one at 15.50 eV in the spectrum of RuO_4. These bands were subsequently shown to be from an impurity.

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0020-1669/92/1331-1588$03.00/0