

Bond Valence Sum Analysis of Metalloenzymes. 3. Predicting Bond Lengths in Adjacent Redox States Using Inner-Sphere Reorganizational Energies

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In transporting and delivering electrons needed for catalysis, biological systems often utilize electron-transfer proteins that contain a transition metal center at the active site.^{1–3} Transition metal ions are often stable in two adjacent redox states, and the energy difference can be finely tuned by the ligand environment provided by the protein, allowing precise control over the thermodynamics of electron transfer. Electron-transfer kinetics are favorable with transition metals because the changes in the metal–ligand bond lengths are relatively small compared to those in organic systems, which lowers the barrier to redox interconversion.

The fundamental assumption of electron-transfer theory is that the reactions are simple enough that a relationship between the kinetics and thermodynamics of the reaction can be derived.^{1–3} Specifically, the shapes of the potential energy surfaces of the initial and final states must be similar enough that the transition state is halfway along the reaction coordinate at zero driving force. This situation allows the calculation of the activation free energy from the driving force and the vertical energy difference between the initial and final states, which is called the reorganizational energy. This energy is composed of an outer-sphere solvent reorganization term (usually the larger term) and the inner-sphere reorganizational energy, λ_i , which is the sum of squares of the changes in bond length between the redox states times the force constant for each bond:³

$$\lambda_i = \sum \frac{1}{2} k_j (\Delta x_j)^2 \quad (1)$$

where k_j is the force constant for the j th bond and Δx_j is the change in the length of the j th bond between the two redox states. The appeal of electron-transfer reactions and the associated theory is that the ground-state geometries (i.e., Δx_j) can be used to calculate kinetic parameters (i.e., λ_i), a feature that is exploited here.

There has been considerable interest recently in using bond valence sums (BVS) to assess the compatibility of a given set of metal–ligand bond lengths with the assignment of a particular oxidation state to a central metal atom in a coordination complex.^{4–10} The BVS concept was originally developed by Brown for analysis of the crystal structures of solids.^{11,12} Calculation of the BVS involves using the relationship for the valence (V_j) of a given bond as

$$V_j = \exp[(r_o - r)/B] \quad (2)$$

where r is the length of a given bond, B is a constant at 0.37 Å,

and r_o is an empirical parameter for a given metal–ligand combination. The sum of the individual values of V_j for the j bonds around a central metal gives the BVS, which is generally close to the formal oxidation state of the metal; this notion can be traced originally to Pauling in 1929.¹³ The BVS analysis is useful for interpreting EXAFS data on metalloproteins where bond lengths are determined with high precision but where coordination numbers are usually not uniquely defined in the fit.¹⁴ By determining how many multiples of V_j for each type of bond are required to give the formal oxidation state, the coordination number can also be determined.^{6,7} This analysis has been frequently applied to interpreting EXAFS data on metalloproteins,^{15–20} understanding changes in metal–ligand bond lengths about zinc ions during hydrolytic catalysis,^{21,22} and in analyzing geochemical substances.²³

Upon reduction, the metal–ligand bonds in a coordination complex generally lengthen to compensate for the lower positive charge developed on the metal center by the addition of an electron. In homoleptic complexes, the BVS method could be used directly to calculate the new bond length provided all of the bonds lengthened by the same amount, since a unique value of r would give a V_j equal to the new oxidation state divided by the number of bonds. In heteroleptic complexes, however, the analysis is not straightforward. Even in a complex with only two different types of bonds, there are many combinations of expansions of one bond or the other that would cause the BVS to decrease by 1.0 from a given starting point. For example, one type of bond could lengthen while the other remained constant or both could lengthen by an equal amount or any other combination (including expansion of one bond and contraction of the other) that would increment the BVS appropriately. Here I show that the bond lengths in the reduced state can be predicted if the combination of bond length changes that give the minimum λ_i according to eq 1 is selected from the subset of bond length changes that give the appropriate BVS value. These calculations can be performed by hand or using a few lines of code on a minimal computer or calculator.²⁴

- (4) Garner, C. D. *Physica B* **1995**, 209, 714–716.
- (5) Hati, S.; Datta, D. *J. Chem. Soc., Dalton Trans.* **1995**, 1177–1182.
- (6) Thorp, H. H. *Inorg. Chem.* **1992**, 31, 1585.
- (7) Liu, W.; Thorp, H. H. *Inorg. Chem.* **1993**, 32, 4102–4105.
- (8) Palenik, G. J. *Inorg. Chem.* **1997**, 36, 4888–4890.
- (9) Palenik, G. J. *Inorg. Chem.* **1997**, 36, 3394–3397.
- (10) Palenik, G. J. *Inorg. Chem.* **1997**, 36, 122.
- (11) Brown, I. D. *J. Solid State Chem.* **1989**, 82, 122.
- (12) Brown, I. D.; Altermatt, D. *Acta Crystallogr.* **1985**, B41, 244.
- (13) Pauling, L. *J. Am. Chem. Soc.* **1929**, 51, 1010–1026.
- (14) Cramer, S. P.; Hodgson, K. O. *Prog. Inorg. Chem.* **1979**, 25, 1–40.
- (15) Bell, J.; Ash, D. E.; Snyder, L. M.; Kulathila, R.; Blackburn, N. J.; Merkler, D. J. *Biochemistry* **1997**, 36, 16239–16246.
- (16) Butler, A.; Clague, M. J. *Adv. Chem. Ser.* **1995**, 246, 329–349.
- (17) Mahapatra, S.; Halfen, J. A.; Wilkinson, E. C.; Pan, G. F.; Wang, X. D.; Young, V. G.; Cramer, C. J.; Que, L.; Tolman, W. B. *J. Am. Chem. Soc.* **1996**, 118, 11555–11574.
- (18) Murphy, L. M.; Strange, R. W.; Hasnain, S. S. *Structure* **1997**, 5, 371–379.
- (19) Scarrow, R. C.; Brennan, B. A.; Cummings, J. G.; Jin, H. Y.; Duong, D. H. J.; Kindt, J. T.; Nelson, M. J. *Biochemistry* **1996**, 35, 10078–10088.
- (20) Shu, L. J.; Chiou, Y. M.; Orville, A. M.; Miller, M. A.; Lipscomb, J. D.; Que, L. *Biochemistry* **1995**, 34, 6649–6659.
- (21) Xiang, S.; Short, S. A.; Wolfenden, R.; Carter, C. W., Jr. *Biochemistry* **1995**, 34, 4516–4523.
- (22) Xiang, S. B.; Short, S. A.; Wolfenden, R.; Carter, C. W., Jr. *Biochemistry* **1996**, 35, 1335–1341.
- (23) Xia, K.; Bleam, W.; Helmke, P. A. *Geochim. Geophys. Acta* **1997**, 61, 2211–2221.

- (1) Gray, H. B.; Ellis, W. R., Jr. In *Bioinorganic Chemistry*; Bertini, I., Gray, H. B., Lippard, S. J., Valentine, J. S., Eds.; University Science Books: Mill Valley, CA, 1994; pp 315–364.
- (2) Lippard, S. J.; Berg, J. M. *Principles of Bioinorganic Chemistry*; University Science Books: Mill Valley, CA, 1994.
- (3) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, 811, 265.

Table 1. Parameters Used in Calculations on Copper and Diiron Proteins

	r_o (Å) ^a	k (kcal/mol) ^b		r_o (Å) ^a	k (kcal/mol) ^b
Cu ²⁺ -N	1.751	388	Fe ³⁺ -O	1.759	485
Cu ⁺ -N	1.595	388	Fe ²⁺ -O	1.734	485
Cu ²⁺ -S	2.054	336	Fe ³⁺ -N	1.855	419
Cu ⁺ -S	1.898	336	Fe ³⁺ -N	1.806	419

^a Taken from ref 6. ^b Calculated according to ref 25; force constants for Fe are for oxidized MMO and are slightly different for Hr according to eq 3.

Parameters

The r_o values used were those reported in an earlier paper⁶ and are given in Table 1 for the specific cases of interest here. There has been considerable discussion recently of how best to determine the r_o parameters.^{5,7} Palenik has discussed the importance of developing oxidation-state independent r_o values,⁸⁻¹⁰ which could be very powerful for determining oxidation states or predicting absolute bond lengths. Since the present calculations are aimed at determining changes in bond length rather than absolute bond lengths, the distinctions associated with oxidation-state independent parameters are not particularly important, so I have chosen to use our original oxidation-state dependent r_o values as set out in Table 1. In the applications discussed here, the oxidation states are known explicitly, so the oxidation-state dependent values are adequate.

To use eq 1 in the minimization, values of the force constants for the important metal-ligand bonds are required. I have chosen to use force constants calculated according to the methods and parameters of the universal force field (UFF) described by Rappé et al.²⁵ These force constants are determined using an approach similar to Badger's rule as

$$k = 664.12Z_1Z_2/r^3 \quad (3)$$

where Z_1 and Z_2 are charge parameters specific for each element in the bond and r is the equilibrium bond length. The bond length chosen for r was that present in the starting (oxidized) state; the force constants used are given in Table 1. Although eq 3 predicts a change in force constant in the two states (which have different r values), this change is reasonably small (~10% for a 0.1 Å change in bond length). Further, the predicted bond lengths will be influenced not by the absolute force constants but rather by the relative force constants of the two bonds being optimized in the systems described. If both bonds are changing in the same direction, the effect of the changing force constants is negligible. This assumption was confirmed by performing the calculations using either the force constants for the initial state, the force constants for the final state, or the average of the initial and final force constants. In the systems studied here, all three calculations gave the same optimum bond lengths. This condition is important since an assumption of electron-transfer theory is that the potential energy surfaces have the same shape in both the initial and final states.¹⁻³

Plastocyanin

The first system chosen was plastocyanin because X-ray crystallography and EXAFS have been performed on both the Cu(I) and Cu(II) states.²⁶ The initial state in all of the cases discussed here was the oxidized form, which was the Cu(II) state for plastocyanin. The coordination of copper in plasto-

cyanin is by two histidines and a thiolate from cysteine. The long bond to the thioether gave a very small V_j and was neglected. The observed distances by EXAFS are an average distance for the two nitrogens of $r_N(+1) = 2.05$ Å, $r_N(+2) = 1.97$ Å and a unique distance for the thiolate of $r_S(+1) = 2.21$ Å, $r_S(+2) = 2.05$ Å. As discussed in an earlier paper,⁶ these distances give good BVS values for the +1 and +2 states of 1.02 and 1.95, respectively.

The calculation involved determining all of the BVS and λ_i values for each combination of bond lengths beginning with the starting (oxidized) state and incrementing each bond by 0.01 Å from the starting distance up to the starting distance + 0.20 Å. For plastocyanin, therefore, BVS and λ_i values were calculated for each combination where the $1.97 < r_N < 2.17$ Å and $2.11 < r_S < 2.31$ Å for a total of 441 states. The BVS for each state was then $2 \exp((1.595 - r_N)/0.37) + \exp((1.898 - r_S)/0.37)$. There were 101 states with a BVS between 0.96 and 1.04, and these states had λ_i values ranging from 3.4 to 15.5 kcal/mol. There was at least one state with an acceptable BVS for every increment of each bond. For example, the state where r_N was unchanged but there was an unreasonably long r_S (2.38 Å) had a λ_i of 12.3 kcal/mol. The state with an unchanged r_S and an elongated r_N (2.16 Å) had a λ_i of 7.0 kcal/mol. The maximum energy state had a $\lambda_i = 15.5$ kcal/mol, $r_N = 2.17$ Å, and $r_S = 2.12$ Å. The state with the minimum energy had $r_N(+1) = 2.03$ Å and $r_S(+1) = 2.22$ Å, which compared well with the experimental values.

There were 14 states that had both acceptable BVS values and λ_i values that were within 1 kcal/mol of the minimum energy. These states were found for bond distances of $r_N(+1) = 2.00$ – 2.06 Å and $r_S(+1) = 2.18$ – 2.25 Å. These ranges included the precise experimental distances observed of $r_N(+1) = 2.05$ Å and $r_S(+1) = 2.21$ Å. Minimization of the energy without selection of acceptable BVS values did not produce a meaningful result, because the absolute minimum energy for the entire set of 441 states is simply the state where $r(+1) = r(+2)$ and $\lambda_i = 0$. Thus, the BVS allows for the selection of the subset of chemically reasonable states from which the minimum energy state can be chosen.

The systems described here are all ones in which the EXAFS analysis gives two distinct bond distances, which are often average distances for two or more types of bonds. In plastocyanin for example, the EXAFS analysis gives an average distance for two Cu-N bonds and a unique distance for the Cu-S bond. Within the states examined, there are of course a host of other states where, for example, the two Cu-N bonds in plastocyanin differ from the average by varying amounts. The approach here does not attempt to differentiate between these states, primarily because the experimental data do not. As the number of unique bonds increases, the number of states with low inner-sphere reorganizational energies and compatible BVS values also increases, so the analysis is best used to treat the minimal number of states needed to be informative. The analysis is applicable to more unique bonds simply by adding terms to eqs 1 and 2; however, the number of compatible states will increase. This issue has been discussed in detail in earlier papers.^{6,7}

Dinuclear Iron Proteins

Calculations similar to that for plastocyanin were performed for the diiron centers in the methane monooxygenase (MMO) and hemerythrin (Hr) proteins.²⁷ For MMO, the two distinct

(24) Calculations were performed in Microsoft Visual Basic 5.0 on a 133 MHz Pentium computer. Programs consisted of nested FOR/NEXT loops for each bond minimized.

(25) Rappé, A. K.; Casewit, C. J.; Colwell, K. S.; Goddard, W. A., III; Skiff, W. M. *J. Am. Chem. Soc.* **1992**, *114*, 10024–10035.

(26) Sykes, A. G. *Chem. Soc. Rev.* **1985**, *14*, 283 and references therein.

(27) Feig, A. L.; Lippard, S. J. *Chem. Rev.* **1994**, *94*, 759–805.

Table 2. Experimental and Predicted Distances (in Å) in Copper and Diiron Proteins

	experimental				predicted reduced	
	oxidized		reduced		r_1	r_2
	r_1	r_2	r_1	r_2		
Cu ^{2+/+} plastocyanin ^a	1.97	2.11	2.05	2.21	2.03	2.22
Fe ^{3+/2.5+} MMO ^b	1.97	2.14	1.99	2.20	1.99	2.17
Fe ^{3+/2+} MMO ^b	1.97	2.14	2.07	2.24	2.06	2.22
Fe ^{3+/2.5+} Hr ^c	1.79	2.13	1.86	2.13	1.85	2.17

^a Experimental distances taken from ref 26; r_1 is Cu–N, r_2 is Cu–S. ^b Experimental distances taken from ref 28; r_1 is Fe–O, r_2 is Fe–N. ^c Experimental distances taken from ref 29; r_1 is a short Fe–O bond, r_2 is an average Fe–O/N bond, see text.

bonds observed by EXAFS are an Fe–O bond length of 1.97 Å and an Fe–N bond length of 2.14 Å.²⁸ With a coordination model of three Fe–O bonds on each center and two Fe–N bonds on one center and three Fe–N bonds on the other, good BVS values are calculated for the oxidized (BVS = 2.85), semimet (BVS = 2.49), and reduced (BVS = 2.07) states, as discussed previously.⁶

Using the approach described above, the bond distances were calculated for the semimet and reduced forms. For the reduced state, there were seven of the 441 states that had acceptable BVS values and were within 1 kcal/mol of the minimum energy. The minimum energy state was that with $r_N = 2.22$ Å and $r_O = 2.06$ Å, which was in good agreement with the observed experimental distances of $r_N = 2.24$ Å and $r_O = 2.07$ Å. The same result was obtained by starting with the fully oxidized distances and minimizing the energy of the fully reduced state based on a electron change at each metal center or by starting with the semimet state and reducing the Fe(III) center. For the semimet form, the calculation was performed by starting with the oxidized state and reducing a single Fe(III) center. There were 9 of the 441 states that had acceptable BVS values and were within 1 kcal/mol of the minimum, and the minimum energy state gave $r_O = 1.99$ Å and $r_N = 2.17$ Å, which was in good agreement with the experimental distances of 1.99 and 2.20 Å.

Calculations were also performed for Hr starting with the oxidized form and calculating the distances for the semimet form. The coordination model for the oxidized state was one short Fe–O bond on each metal center at 1.79 Å and two long Fe–O bonds and three Fe–N bonds at 2.12 Å.²⁹ For the calculated semimet form, there were six states with acceptable BVS values and energies within 1 kcal/mol of the minimum energy. The minimum energy state gave a short $r_O = 1.85$ Å and a long $r_{O/N} = 2.17$ Å, in good agreement with the observed distances of 1.86 and 2.13 Å. Results for the calculations on plastocyanin and the diiron proteins are summarized in Table 2.

Discussion

The BVS analysis has been widely applied to problems in coordination chemistry, particularly in the assignment of oxidation states and coordination numbers in the interpretation of EXAFS data^{4–7} but also in interpretation of more complex phenomena such as the interpretation of bond length changes during hydrolytic catalysis.^{21,22} The BVS method is a very simple means for assessing the degree of charge transfer between Lewis acid metal centers and bound donor atoms, a problem

that is of central importance in developing force fields for metal atoms.³⁰ This problem has been addressed using either an iterative method for assigning partial charges to metal ions and ligands³¹ or using a valence bond description of the entire electronic structure of the metal–ligand bonds.³² These approaches are much more complete and offer means for predicting absolute bond lengths and bond angles, whereas here we use the BVS method only for selecting compatible subsets of bond distances that must then be optimized using some energy function. Nonetheless, the simplicity of the BVS method has resulted in its widespread routine use, and application to other problems in coordination chemistry as described here is therefore attractive.

As stated above, the idea that the bond valences should sum to the oxidation state was originally proposed by Pauling.¹³ Further, the idea that changes in the ground-state metal–ligand distances could be used to predict the minimum transition state energy for electron transfer can be attributed to early work by Marcus and others.³ These two ideas combine nicely here for predicting bond distances in neighboring redox states. These findings suggest that, at least in the systems tested here, the conformational freedom in the folded protein is sufficient relative to the small changes in metal–ligand bond distance that the optimum configurations can be obtained. In these systems, there are well-defined sets of bond distances that optimize both changes in electron density and inner-sphere reorganizational energy, and these optimum conditions can apparently be met by the host protein.

Despite the good agreement in Table 2, the considerations described here do not necessitate optimized values of λ_i and V_j . Rather, the approach provides a simple means for assessing the compatibility of the bond lengths in the two redox states and the implied inner-sphere reorganization. If the force constants are very different in the two states, then the assumption of electron-transfer theory that the two potential energy surfaces are the same does not hold, which would lead to a deviation from the optimum bond lengths. Further, if V_j is not optimum in one of the two states because of steric constraints in the protein, the calculated λ_i will not be meaningful. Such a system may more likely be found in rigid small molecule complexes where steric constraints are more important. So although the approach can be used to predict the bond lengths in an adjacent redox state in a system where the bond lengths can only be measured in one of the two states (with the assumptions that λ_i and V_j are optimized), the approach can alternatively be used to test whether λ_i and V_j are optimized in a protein where the bond lengths are known in both states, as was essentially done here. A system where the protein conformation forces the selection of an unoptimized set of bond distances by these criteria has not yet been identified, although the existence of such a system can be supported using the relationship between the BVS values and λ_i .

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(28) DeWitt, J. G.; Rosenzweig, A. C.; Salifoglou, A.; Hedman, B.; Lippard, S. J.; Hodgson, K. O. *Inorg. Chem.* **1995**, *34*, 2505–2515.
 (29) Scarrow, R. C.; Maroney, M. J.; Palmer, S. M.; Que, L., Jr.; Roe, A. L.; Salowe, S. P.; Stubbe, J. *J. Am. Chem. Soc.* **1987**, *109*, 7857.

(30) Zimmer, M. *Chem. Rev.* **1995**, *95*, 2629–2649.
 (31) Vedani, A.; Huhta, D. W. *J. Am. Chem. Soc.* **1990**, *112*, 4759–4767.
 (32) Allured, V. S.; Kelly, C. M.; Landis, C. R. *J. Am. Chem. Soc.* **1991**, *113*, 1–12.