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Angular Overlap Interpretation of the Spectromagnetic Properties of the Nitrate Derivative of Cobalt(II)-Substituted Carbonic Anhydrase

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We have applied the angular overlap model of the ligand field (AOM) to reproduce the d–d electronic transitions, the *g* values and, in particular, the magnetic susceptibily tensor, χ, of bovine cobalt-substituted carbonic anhydrase reacted with nitrate ($CoCANO_3$). The room-temperature magnetic susceptibily tensor has been previously found by measuring the pseudocontact shift of a number of protons via ^{1}H-NMR spectroscopy. The results are satisfactory and provide a new route for the determination of the ligand field parameters of paramagnetic metal ions in metalloproteins.

Introduction

The angular overlap model (AOM) of the ligand field[1] has been widely applied with success since 1975 to the interpretation of the spectromagnetic properties of transition metal ions in low-symmetry environments.[2-5] In particular, several high-spin cobalt(II) complexes have been studied in a number of different stereochemistries and the observed d–d transitions and magnetic and hyperfine tensors (χ, **g**, **A**) have been reproduced by the calculations.[4] In general the number of parameters required by the model is larger than the number of spectroscopic observables and single-crystal measurements have been used to increase this number. However, some parameter must be still kept as fixed. In metalloproteins only solution data are in general available; therefore, the number of spectroscopic data is in general too low to attempt any theoretical approach. In proteins containing a paramagnetic metal ion, however, the ^{1}H-NMR shifts of protons close to the paramagnetic metal but separated by several chemical bonds are pseudocontact in origin and are related to the magnetic anisotropy and to the orientation of the χ tensor of the paramagnetic center.[6,7] Through NMR, therefore, new information can become available which allow one to use a ligand field model to describe the electronic structure of the metal ion.

Recently, the magnetic susceptibility tensor, χ, of the nitrate derivative of the cobalt-substituted bovine carbonic anhydrase, $CoCANO_3$, was estimated by fitting the pseudocontact shifts of several protons in key positions.[6] A schematic view of the coordination environment of the cobalt(II) ion in the protein is shown in Figure 1. The cobalt center is five coordinated, in a geometry intermediate between a trigonal bipyramid and a square pyramid. The donors are three nitrogen and two oxygen atoms. The three nitrogen donors come from three different histidine residues, and the other two coordination sites are occupied by one water molecule and one donor atom of the coordinated anion. The principal directions of the χ tensor make angles of 39, 164, and 32° with the Co–N(His-94), Co–N(His-119), and Co–anion bonds, respectively (see Figure 1). The axial and equatorial magnetic anisotropies, measured at room temperature, were found to be $\Delta\chi_{ax} = \chi_{zz} - {}^1/_2(\chi_{xx} + \chi_{yy}) = -6 \times 10^{-8}$ m^3 mol^{-1} and $\Delta\chi_{eq} = (\chi_{xx} - \chi_{yy}) = 3 \times 10^{-8}$ m^3 mol^{-1}, where the *z* direction was assumed to be the closest to the Co–anion bond direction.[6] The error on the above values is rather large because there is some uncertainty in the evaluation of the pseudocontact shifts due to the lack of reference shifts in the large diamagnetic zinc protein. The order of magnitude of the magnetic anisotropies is nevertheless correct, and the magnetic directions can be accurate within 15°. Due to the low-symmetry environment of the coordination site of cobalt(II) in the enzyme, no perturbation procedure seemed easily applicable to reproduce these data and we resorted to perform AOM calculations using full matrix diagonalization.[4] One of the advantages of this method is that it allows one to simultaneously compute the largest possible number of spectroscopic observables, like d–d electronic transitions and **g** and χ tensors, and to optimize the large number of parameters required by the model.

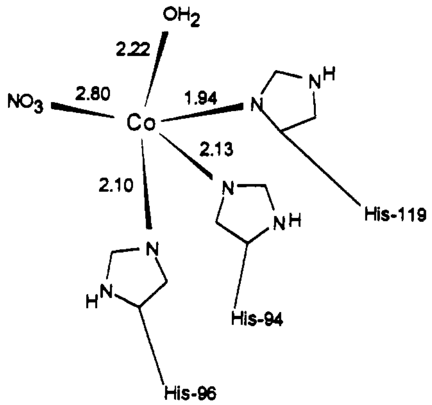

Figure 1. Schematic structure of the $CoCANO_3$ chromophore (bond distances in Å).

In the following we wish to report the results of our calculations on $CoCANO_3$, whose visible[8] and EPR spectra[9] have been previously measured.

Computational Details

The calculations have been performed using the computer programs previously described.[3,4] All the calculations have been performed on IBM 9370/50 and IBM Personal System 2 80/110 computers.

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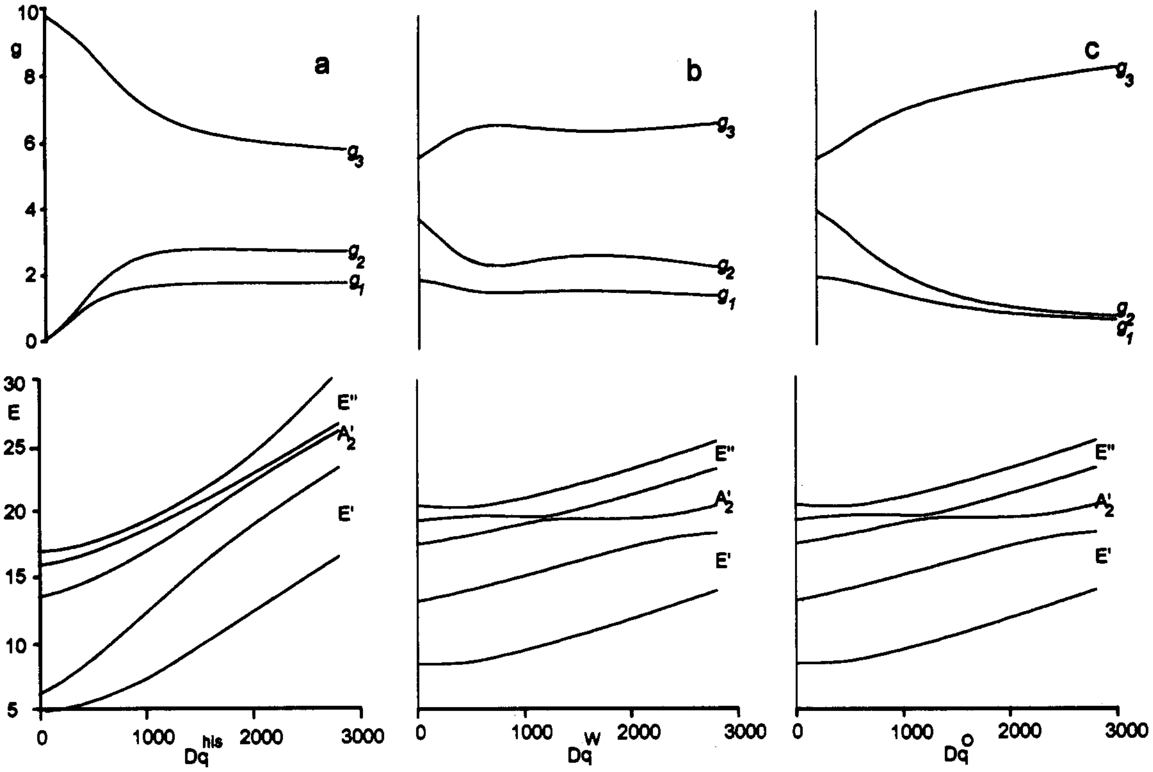

Figure 2. Calculated transition energies (cm^{-1} × 10^3; bottom) and g values (top) with B = 825 cm^{-1}, k = 0.90, ζ = −533 cm^{-1}: (a) effect of varying the Dq^{his} parameters (Dq^{w} = 1200 cm^{-1}, Dq^{o} = 500 cm^{-1}); (b) effect of varying Dq^{w} (Dq^{his} = 1300 cm^{-1}, Dq^{o} = 500 cm^{-1}); (c) effect of varying Dq^{o} (Dq^{his} = 1300 cm^{-1}, Dq^{w} = 1200 cm^{-1}). In the three cases for the histidine, H_2O, and NO_3 ligands, respectively, the following e_π/e_σ parameters have been used: 0.13, 0.05, 0.05.

The calculations have been performed on a CoN_3OW (W = oxygen of H_2O) chromophore with the angular coordinates seen in the crystal structure.[10] The angular coordinates, θ, φ, and ψ, for each ligand atom are collected in Table I and referenced to the x, y, z Cartesian reference system[1] defined in ref 6. These coordinates correspond to the Euler angles[11] β, γ, and α, respectively.

The π-antibonding effect of the oxygen atoms of the nitrate ion and of the water ligand can be considered isotropic. Therefore, in the AOM, their overall antibonding interactions are described by four parameters, e_σ^{o}, e_π^{o}, e_σ^{w}, and e_π^{w} for the nitrate and the water oxygen, respectively. The π-antibonding interaction of the histidine ligands is due to an almost N nonbonding orbital perpendicular to the ligand plane; therefore, two parameters are generally required to describe this interaction which depend on the angle ψ between the π direction and the zz' plane,[4] z' being the Co–N bond direction. The two π parameters are defined according to (1). When ψ = 0° the histidine plane is perpendicular to the zz' plane

$$e_{\pi s}^{N} = (\sin^2\psi)e_\pi^{N} \qquad e_{\pi c}^{N} = (\cos^2\psi)e_\pi^{N} \qquad (1)$$

and only one parameter, $e_{\pi c}^{N}$, is required to describe the π antibonding interaction. Using the crystallographic data the ratios $e_{\pi s}^{N}/e_{\pi c}^{N}$ of 0.37, 0.052, and 1.3 were computed for His-96, His-94, and His-119, respectively. These values were kept constant in the calculations.

With application of the above constraints to the parameters set, the total number of free bonding parameters is 10. In order to further reduce the number we treated His-94 and His-96 in the same way (the last two ligands bind the metal using N-ϵ_2, while His-119 binds the metal with the N-δ_1 ligand atom), thus reducing the number of bonding parameters to 8. The spin–orbit coupling constant, ζ, and the orbital reduction factor, k, are also parameters to vary, but we have kept them fixed to values generally used in the literature, −533 cm^{-1} and 0.9, respectively. This approximation will prevent us from obtaining an exact numerical reproduction of the magnetic observables; therefore, we will not put all the attention on the principal values of the magnetic tensors but rather to their principal directions. Also, instead of giving a set of best fit parameters, we will show the effect that the parameters can have on the magnetic and spectroscopic observables in order to determine a range of variability of them around a possible best fit value. In the calculations we generally used the ligand function (2) instead of the e_σ^{L} and e_π^{L}

$$10Dq^{L} = (3e_\sigma^{L} - 2e_{\pi s}^{L} - 2e_{\pi c}^{L}) \qquad (2)$$

separately for any L ligand. When all the ligands are equal, this value represent the usual Dq parameter of the symmetry-dependent parameterization of the ligand field.

Table I. Geometrical Coordinates for the Donor Atoms in Carbonic Anhydrase Chromophores

ligand	θ	φ	ψ
N(His-94)	118.7	245.3	12.79
N(His-96)	26.90	315.0	31.19
N(His-119)	68.42	143.5	131.4
W	109.3	359.3	0
O	148.9	82.34	0

Only quartet states were included in the calculations.

Results

In order to know the effect of the AOM parameters on the spectroscopic observables, sample calculations have been performed by varying the bonding parameters over a wide range. These calculations are required for evaluating the uncertainties on a set of best fit parameters and to evaluate a possible range of existence of a parameter. Selected results of these calculations are shown in Figures 2 and 3.

In Figure 2 (bottom) the dependence of the electronic transitions with energies higher than 5000 cm^{-1} on the Dq^{L} parameters of histidine ligands (Figure 2a, bottom), water (Figure 2b, bottom), and the nitrate ion (Figure 2c, bottom) is shown. The curves are labeled according to the symmetry of the excited state in D_{3h} symmetry, corresponding to a regular trigonal bipyramidal coordination. In this limit, the ground state is $^4A_2'$. The next excited states (not shown) can be labeled as $^4A_1''$+$^4A_2''$ and $^4E''$.

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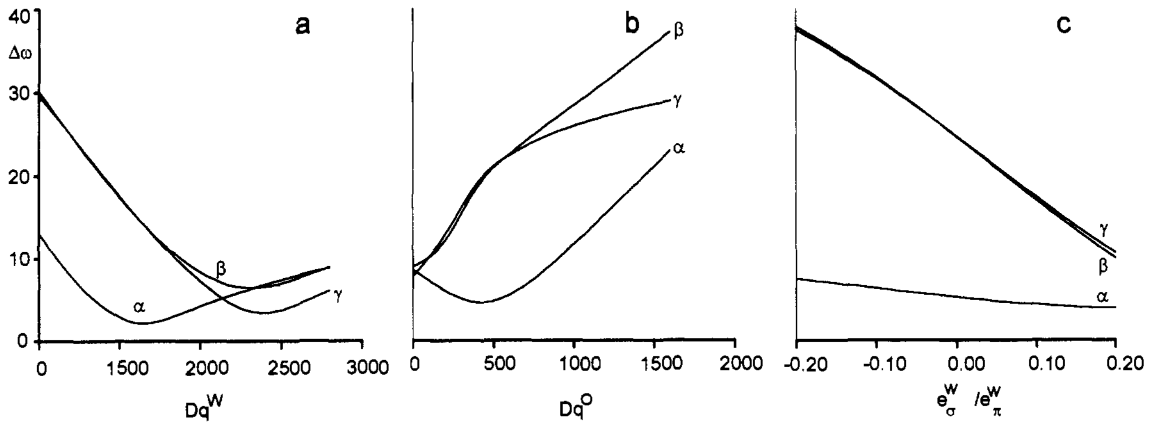

Figure 3. Angles ($\Delta\omega^{\circ}$) between the computed principal directions of χ and the observed ones:[6] (a) effect of varying Dq^{w} (Dq^{his} = 1300 cm^{-1}, Dq^{o} = 500 cm^{-1}, parameters e_{π}/e_{σ} on the three type of ligands 0.05, 0.13, and 0.05, respectively); (b) effect of varying Dq^{o} (Dq^{his} = 1300 cm^{-1}, Dq^{w} = 1200 cm^{-1}, parameters e_{π}/e_{σ} unchanged); (c) effect of varying $e_{\pi}{}^{w}/e_{\sigma}{}^{w}$ (Dq^{his} = 1300 cm^{-1}, Dq^{w} = 1200 cm^{-1}, Dq^{o} = 500 cm^{-1}, $e_{\pi}{}^{his}/e_{\sigma}{}^{his}$ = 0.13, $e_{\pi}{}^{o}/e_{\sigma}{}^{o}$ = 0.05).

It is apparent that the histidine ligands have the largest influence on the electronic energies.

In Figure 2 (top) the computed dependence of the principal g values on the Dq^{L} parameters are shown. Only histidines (Figure 2a, top) and the nitrate ion (Figure 2c, top) significantly affect these values. All the above observables are very little influenced by the e_{π}/e_{σ} values of the ligands. The principal values of the χ tensor closely follow the behavior of the principal values of the **g** tensor. The principal directions of the **g** and χ tensors were found to be almost coincident (<5°) in all the calculations. They were found almost unaffected by the variation of Dq^{N}, while they showed a marked dependence on $e_{\pi}{}^{w}/e_{\sigma}{}^{w}$.

In Figure 3 the dependence of the angles between the computed principal directions of χ and the observed ones are shown as function of Dq^{w}, Dq^{o}, and $e_{\pi}{}^{w}/e_{\sigma}{}^{w}$. We have estimated an experimental error on the principal directions of χ of about 15°. We can see, from Figure 3a, that there exist a rather wide range of Dq^{w} values, about 1500 cm^{-1}, in which the angles between principal directions of χ fall within a cone of 15°. The range of Dq^{o} values results much smaller, about 500 cm^{-1} as Figure 3b shows. The principal directions of the χ tensor show a strong dependence on $e_{\pi}{}^{w}/e_{\sigma}{}^{w}$ parameter as we can see in Figure 3c. Fixing the latter parameter to the value of 0.15, in order to have a nice reproduction of these directions, we can estimate a range of "best fit" parameters as the following: Dq^{his} = 1500 ± 200 cm^{-1}; Dq^{w} = 1000 ± 200 cm^{-1}; Dq^{o} = 500 ± 200 cm^{-1}. The Dq values for histidine and water are in the range usually observed for these ligands,[12] and the rather low Dq value for the nitrate ion accounts for the long bond distance (2.8 Å).

A sample fit of the spectroscopic observables is shown in Table II. Using the parameter values reported in Table II, the angles between the calculated principal directions of χ tensor and the observed one result as follows: 4, 5, and 10° for x, y, and z, respectively. The calculated magnetic anisotropies choosing the z axis to be close to the Co–anion bond, as done before,[6] are as follows: $\Delta\chi_{ax} = \chi_{zz}\ {}^{1}/_{2}(\chi_{xx} - \chi_{yy})$ = 3.52 × 10^{-8} m^{3} mol^{-1}; $\Delta\chi_{eq} = \chi_{xx} - \chi_{yy}$ = 2.52 × 10^{-8} m^{3} mol^{-1}. The numerical value of the magnetic anisotropies is strongly influenced by the value of ζ and k, and with our choice of values, we have succeeded in reproducing their order of magnitude. The computed principal directions of the χ tensor agree within experimental errors with the observed ones.

Table II. Best Fit[a] of the Spectromagnetic Properties of CoCANO$_3$

Electronic Transitions (cm^{-1})

obsd[8]	computed	obsd[8]	computed
8 600	8 351[b]	18 000	18 355
10 200	8 945	21 100	19 956
14 200	14 453		20 734

g Values

obsd[9]	computed	obsd[9]	computed
6.4	6.5	1.7	1.7
2.4	2.5		

Magnetic Anisotropies (10^{-8} m^{3} mol^{-1})

obsd[6]	computed	obsd[6]	computed
$\Delta\chi_{ax} = -6$	−3.52	$\Delta\chi_{eq} = 3$	2.52

[a] Best fit parameters: B = 825 cm^{-1}, k = 0.90 cm^{-1}, ζ = −533 cm^{-1}, Dq^{his}(94 and 96) = 1425 cm^{-1}, Dq^{his}(119) = 1500 cm^{-1}, Dq^{w} = 1000 cm^{-1}, Dq^{o} = 400 cm^{-1}, $e_{\pi}{}^{his}/e_{\sigma}{}^{his}$(all) = 0.13, $e_{\pi}{}^{w}/e_{\sigma}{}^{w}$ = 0.15, $e_{\pi}{}^{o}/e_{\sigma}{}^{o}$ = 0.05. [b] Transition coming from lower states not shown in Figure 2.

Conclusions

Anions like HS^{-} [10] and sulfonamides[13] bind the metal ion in carbonic anhydrase, replacing the coordinated water molecule. The formation of a hydrogen bond with the OH group of Thr-199 plays a further driving force to bind in this position. There is spectroscopic evidence that the anions CN^{-} and NCO^{-} behave in similar manner in solution,[14] but X-ray data in the solid state indicate that the two ions do not bind directly the metal.[15] Anions like CH$_3$COO^{-} [16,17] and HCOO^{-} [17,18] bind the metal ion in the fifth position leading to pentacoordinate chromophores. One of the two oxygen atoms binds the metal while the other one interacts with the peptidic NH or Thr-199 through a hydrogen bond.[18] The methyl group (or the hydrogen atom in the case of formate) sits in the hydrophobic pocket of the protein providing contacts with Val-121 and Val-143. Nitrate behaves in the same way displaying a further hydrogen bond with the coordinative water. NCS^{-} [13] and I^{-} [19] are found to bind in the same position indicating a rather general tendency to bind in the hydrophobic pocket. HCO$_3^{-}$, which is the natural substrate, possibly binds both at the

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fifth position and at the water site giving rise to a chemical equilibrium between two species.[20-22]

In this paper we have investigated the bonding mode of NO_3^- by fitting d–d transitions, *g* values, and the magnetic susceptibility tensor using AOM. Our results show that the bonding interaction of the nitrate anion, estimated by the *Dq* value, is almost half that of the water molecule and $^1/_3$ of that of one histidine ligand. The estimate of the *Dq* is rather sound because its value dramatically affects the directions of the χ tensor. It should be noted that in all cases where five-coordination occurs, the methyl group of the Thr-199 and Thr-200 are invariably shifted by dipolar coupling one upfield and the other downfield, respectively.[22] This means that the magnetic susceptibility tensor does not change much within the series. It appears therefore that the fifth donor is always weak. In other words it appears that the fifth ligand binds in a rather loose way as a result of a balance between the coordination bond, the hydrogen bonds, and the hydrophobic interactions. The X-ray data in the solid state had found zinc–fifth donor distances ranging from 2 to 2.8 Å. Only the thyocyanate ligand shows a Zn–N bond of 2 Å,[13] and indeed the ^1H NMR spectrum of the cobalt(II) analog is at an extreme of the series.[22] We can now propose that a weak fifth donor accounts for the general spectroscopic properties of these five-coordinated adducts.

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