An ab Initio Quantum-Chemical Study of the Blue-Copper Site of Azurin

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Abstract: The electronic structure of the blue-copper site of *Pseudomonas aeruginosa* azurin has been investigated by ab initio multireference determinantal configuration interaction (MRD-CI) calculations. A truncated site consisting of copper and its three equatorial ligands has been studied with emphasis on the **g** tensor and the nitrogen hyperfine tensors of the coordinating histidines. In the ground state the singly occupied molecular orbital (SOMO) involves a copper 3d orbital π antibonded to the cysteine sulfur and σ antibonded to the histidine nitrogens. A proper description of the electron-paramagnetic-resonance parameters has been achieved through the use of an effective core potential for copper up to and including the 3s electrons. Both the complete **g** tensor and the anisotropic hyperfine tensors at the nitrogens are essentially reproduced. Mulliken spin densities of 35 and 59% on copper and sulfur, respectively, and 2.1 and 1.7% on the respective coordinating nitrogens reflect the delocalized character of the SOMO and the inequivalence of the histidines.

Introduction

Blue-copper proteins are characterized by an intense absorption around 600 nm and an unusually small copper hyperfine interaction. The metal site involves a copper that is strongly bound to two histidines and a cysteine at a distance of about 2.1 Å. In most cases, the axial position is taken by the Sδ atom of a methionine at a relatively large distance of about 3 Å, and the structure around copper is close to a trigonal pyramid. Following the crystallization and subsequent X-ray diffraction study of plastocyanin of *Populus nigra* in the early eighties of the last century,[1] this blue-copper protein has become the reference point for quantum-chemical studies[2−7] that aim for understanding the electronic structure of the metal site in this class of proteins. Initial quantum-chemical investigations concerned self-consistent field Xα-scattered wave (SCF-Xα-SW) calculations on sites that model the copper site of plastocyanin.[3] These calculations pointed to a substantial covalency of the metal−ligand bonds and indicated a 40% copper-3d and 36% sulfur-3p$_\pi$ (cysteine) character of the molecular orbital that is

singly occupied (SOMO) in the ground state of the oxidized protein. Later, the calculations were extended to provide a description of optical absorption and MCD and CD spectra.[4] The intense 600 nm absorption was interpreted as a ligand to metal [p$_\pi$ S(Cys) → d$_{xy}$ Cu] "charge-transfer" transition. In these SCF-Xα-SW calculations, the atomic sphere radii were adjusted to reproduce the experimental *g* values.

Following the pioneering work of the Solomon group, various other quantum-chemical approaches to the study of trigonal blue-copper sites have been reported with the emphasis on the reproduction of the electronic absorption spectrum. Use has been made of CNDO/S,[6] CASSCF/CASPT2,[7] and density functional theory.[8] These calculations confirmed the S(Cys) p$_\pi$ to Cu 3d$_{xy}$ nature of the transition that is responsible for the strong absorption in the visible and showed that the charge transferred in this transition is negligible. They differed significantly as regards the energies of the molecular orbitals and the relative spin densities at copper and sulfur in the SOMO. For example, in ab initio Hartree−Fock calculations on a model site of azurin, a protein that contains an even more ideal trigonal copper site than plastocyanin, the SOMO was dominated by either copper or sulfur atomic orbitals.[6] The CNDO/S calculation on the same site led to densities of 32% at copper and 50% at sulfur in the SOMO of the ground state.[6] Largely varying compositions of this orbital were also reported for CASSCF/CASPT2 calculations on trigonal model sites of plastocyanin with C_s symmetry. For Cu(Im)$_2$(SCH$_3$)(S(CH$_3$)$_2$)$^+$ densities on copper and sulfur of 54 and 45% were obtained,[7] for Cu(Im)$_2$(SH)(SH$_2$)$^+$ of 81

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and 17%.[9] More recently, similar quantum-chemical studies have been performed on the copper sites of nitrite reductase, stellacyanin, cucumber basic protein, and laccase.[9−13]

From an experimental point of view, the SOMO is best characterized for the blue-copper protein azurin. An electron-spin−echo (ESE)-detected electron paramagnetic resonance (EPR) study at 95 GHz on a single crystal of azurin has allowed the determination of the complete **g** tensor, that is, the principal values as well as the directions of the principal axes with respect to the copper-ligand directions.[14] In addition, the application of the electron spin−echo envelope modulation (ESEEM) and electron nuclear double resonance (ENDOR) techniques resulted in knowledge of the complete hyperfine tensors of the copper-coordinated[15] and of the remote nitrogens of the histidines.[16,17] While the **g** tensor primarily provides information about the density at copper and sulfur in the SOMO, the hyperfine tensors reveal information about the extension of the SOMO over the copper-coordinated histidines.

In view of the fact that the EPR experiments have provided a data set that allows a critical test of the calculated wave function, we have performed ab initio quantum-chemical calculations on a copper site that models that of azurin. The three equatorial copper ligands were taken into account. The weak axial ligands, not a conditio sine qua non for a blue-copper site,[13] were not included. We report on a multireference determinantal configuration interaction (MRD-CI) study including single and double excitations. A correct spin-density distribution between copper and sulfur is obtained when the core−electron correlation and the relativistic mass velocity and Darwin terms are taken into account by means of an effective core potential. In that case, the calculation provides an adequate description of both the **g** tensor and the hyperfine tensors of the histidine nitrogens.

Methods

The coordinates of the atoms of *P. aeruginosa* azurin at pH 5.5 are taken from the crystallographic data (PDB file 4AZU).[18] The average structure of the molecules in the unit cell is calculated in the following manner. First the four molecules in the asymmetric unit are rotated on top of each other such that their overlap (excluding the hydrogen atoms) is maximum. Then the coordinates of the corresponding atoms are added and divided by four. The average structure is defined in the reference axes system, called XYZ, in which copper is at the origin, the sulfur of the cysteine is on the negative X axis, and the copper-coordinating nitrogens of the imidazoles are equidistant to the XY plane with Z < 0. The model site consists of a copper atom, an SCH_3 group to represent
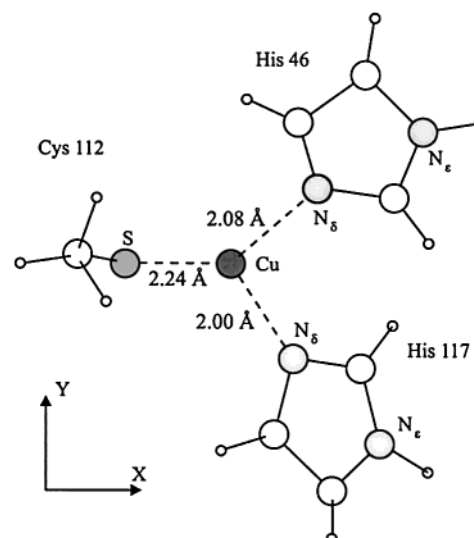

Figure 1. Schematic representation of the $Cu(SCH_3)(N_2C_3H_4)_2$ system used in the calculation as a model for the copper site in *P. aeruginosa* azurin, in which the plane of the paper is the XY plane (cf. Methods).

the cysteine 112, and two imidazoles ($N_2C_3H_4$) to represent the histidines 46 and 117. The hydrogens of the imidazoles are placed at 1.07 Å from carbon and 1.01 Å from nitrogen such that the C−H (N−H) bond direction bisects the angle that involves the carbon (nitrogen) and its neighboring atoms of the ring. The hydrogens of the CH_3 group are placed at 1.10 Å from carbon such that the bond directions from carbon to its neighboring atoms form a tetrahedron. The model site has C_1 symmetry and is presented schematically in Figure 1 (coordinates are available as Supporting Information). In addition, some calculations are performed on model sites with slightly different structures corresponding to those of the four molecules in the asymmetric unit as specified in the PDB file. In the results and discussion section, the SCH_3 and $N_2C_3H_4$ fragments of the model system are referred to by the names of the amino acid residues, which they represent in the copper site of azurin.

The SCF calculations are performed with the MOLCAS 4.0 program package[19] on a closed-shell and neutral system that represents the reduced site. The basis set for copper is the basis set according to Barandiaran,[20] as included in the Molcas 4 basis set library, and used with segmented 8;1, 4;1, and 4;1;1 contractions for s, p, and d valence type functions, respectively. In this basis set core-correlation and relativistic effects are taken into account by an effective core potential (ECP). The effective core includes the copper 1s, 2s, 2p, and 3s electrons. The ECP basis set at copper has been tested in a closed-shell SCF calculation on Cu^+ and an extended basis set marginally improved the energies of the 3d atomic orbitals. The basis set for sulfur is the [13s9p] basis set of McLean and Chandler in the (6s/4p) contraction of Table 16.23.1, for nitrogen the [9s5p] basis set of McLean and Chandler with the (4s2p) contraction of Table 7.62.1, for carbon the [9s5p] basis set of Van Duijneveldt with the (4s2p) contraction of Table 6.61.2, and for hydrogen the [4s] basis set of Huzinaga with the (2s) contraction of Table 1.25.1. Following the closed shell SCF calculation, CI calculations for the oxidized state are performed using the Wuppertal−Bonn MRD-CI[21] with the recent direct CI options of Engels.[22] The 31 electrons in the molecular orbitals are correlated by about 60000 configurations that were selected from all single and double excitations out of a reference set of 35 configurations using a selection

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Table 1. Composition (Mulliken Densities, %) of the Natural Orbitals That Are Singly Occupied in Those Reference Configurations for Which the Coefficient c^2 Is Larger than 0.2 in the Expansion of CI State I (state 0 Corresponds to the Ground State, States 1−9 to the First-Ninth Excited States)

| state | c^2 | Cu | | | | | | | S | | | | Cβ Cys | Nδ 46 | His 46 | Nδ 117 | His 117 |
		s	p	d_{xy}	d_{xz}	d_{yz}	d_{z^2}	$d_{x^2-y^2}$	s	p_x	p_y	p_z					
0	0.69	0	0	21	14	0	0	2	0	0	55	2	1	1	0	1	0
1	0.80	4	6	4	15	0	0	25	0	34	1	7	1	1	0	0	0
2	0.62	0	2	20	38	0	0	30	1	1	2	1	1	1	0	3	1
3	0.80	2	0	0	0	49	36	4	0	1	0	0	4	0	0	0	1
4	0.69	3	1	0	0	40	30	3	1	2	1	8	8	0	0	0	0
5	0.44	0	1	24	5	0	0	0	0	0	16	1	1	0	0	21	31
	0.27	0	0	20	5	0	0	3	0	0	13	1	0	6	6	23	22
6	0.63	2	2	0	19	0	8	24	2	22	0	5	0	6	7	1	3
7	0.30	0	0	0	1	0	0	2	0	2	0	1	0	5	89	0	0
8	0.42	0	0	0	1	0	0	2	0	2	0	1	0	5	89	0	0
	0.31	0	0	1	0	0	1	0	0	1	1	0	0	0	0	5	90
9	0.30	0	0	1	0	0	1	0	0	1	1	0	0	0	0	5	90

[a] For the copper and the sulfur, the densities are given for the s, p, and d basis functions, for the carbon in the model site that represents the Cβ of the cysteine and the nitrogens that represent the δ nitrogens of the histidines the total density is given. The densities denoted by His 46 and His 117 are the sum of the densities at the carbons and the ε nitrogens of the imidazoles that represent the histidines 46 and 117, respectively, and do not include the density at the δ nitrogens.

threshold of 5 μhartree. Energies presented are obtained by using the standard extrapolation and Davidson correction procedures. The CI energies and wave functions are improved by performing an additional MRD-CI calculation with reference configurations based on natural orbitals. The natural orbitals are obtained by diagonalization of an average density matrix which is the sum with equal weight of the density matrixes that correspond to the first three CI wave functions. By including in the density matrix also the contributions of the first two excited states, the bias for the ground state due to the initial use of neutral ground state SCF orbitals is removed and at the same time the convergence of the CI is significantly improved. Test calculations showed that further natural orbital cycles with the same scheme have little effect and indicate that in this way a balanced description of the most important states has been obtained. The energy of the ground state improves approximately 0.025 hartree with respect to that of the CI calculations based on molecular orbitals.

The Mulliken density related to an atomic orbital in a natural orbital is calculated according to

$$2 \sum_\mu \sum_\nu c_\mu c_\nu S_{\mu\nu} \tag{1}$$

where μ runs over the basisfunctions that correspond to the atomic orbital, ν runs over all basis functions, c_μ is the coefficient of the μth basisfunction and S is the overlap matrix.

To calculate the oscillator strengths and the **g** tensor, the transition dipole moments $\vec{\mu}_{ij}$, the orbital angular momentum and spin−orbit-coupling matrix elements between CI states i and j are calculated from the wave functions of the MRD-CI calculation based on natural orbitals. The oscillator strength f_{ij} is obtained according to

$$f_{ij} = \frac{2}{3} \Delta E_{ij} |\vec{\mu}_{ij}|^2 \tag{2}$$

where ΔE_{ij} is the difference in energy of the two states.

The deviation from the free-electron g value stems from spin−orbit coupling. The spin−orbit-coupling corrected wave function is calculated perturbatively with the effective one-electron Hamiltonian

$$H_{SOC} = \sum_i \sum_k \frac{\hbar^2}{2m^2c^2} \frac{Z_k^{eff} e^2}{4\pi\epsilon_0} \frac{\vec{l}_k(i) \cdot \vec{s}(i)}{r_{ik}^3} \tag{3}$$

Here $\vec{l}_k(i)$ represents the orbital angular momentum operator of electron i with respect to nucleus k, $\vec{s}(i)$ the spin angular momentum operator of electron i, r_{ik} the distance of electron i to nucleus k, $\hbar$ the Dirac

constant, m the electron mass, c the velocity of light, e the elementary charge, ϵ_0 the permittivity of vacuum. Spin-other-orbit terms are taken into account by the use of effective nuclear charges. In calculations on small molecules, a value of 65% of the actual nuclear charge is found to be appropriate for copper and 70% for the other nuclei.[23] All matrix elements were calculated using routines in the Wuppertal−Bonn CI program package. The **g** tensor is calculated with an in-house routine according to

$$g_{ij}^2 = 2 \sum_{\mu,\nu=\alpha,\beta} \langle \psi_{SOC}, \mu | L_i + g_e S_i | \psi_{SOC}, \nu \rangle \langle \psi_{SOC}, \nu | L_j + g_e S_j | \psi_{SOC}, \mu \rangle \tag{4}$$

where the sum runs over both states of the Kramer's doublet and g_e is the free electron g value, and $i,j = $ X,Y,Z. Diagonalization of the $\mathbf{g}^2$ tensor yields the g values and the directions of the principal axes (called x, y, and z) of the **g** tensor.

Atomic spin densities are calculated from the spin-density matrix by a Mulliken population analysis. For the anisotropic hyperfine tensors of the nitrogens, the dipolar integrals between the spin of the unpaired electron (described by the spin-density matrix of the CI ground state) and the nuclear spin (fixed at the nucleus) are evaluated.[24] The expression for the complete tensor is given by

$$\vec{\vec{A}} = \frac{\mu_0}{4\pi} g_N \beta_n g_e \beta_e \langle (3\hat{r}\hat{r} - E)/r^3 \rangle \tag{5}$$

In this equation μ_0 is the permeability of vacuum, g_N is the nuclear g value of nitrogen, β_n is the nuclear magneton, β_e is the Bohr magneton, $\hat{r}$ is a unit vector ($\hat{r} = \hat{x}$, $\hat{y}$, $\hat{z}$) and E is the 3×3 unit matrix. The principal axes of the hyperfine tensor are called x'', y'', and z'', where x'' corresponds to the largest principal value ($A_{x''x''}$) and z'' to the smallest principal value ($A_{z''z''}$).

Results

The eigenstates resulting from the MRD-CI calculation on the model of the blue-copper site of azurin are represented in Table 1, where state 0 corresponds to the ground state and states 1−9 to the first to ninth excited states. Each CI wave function is a linear combination of configurations, and each configuration consists of doubly occupied and singly occupied natural orbitals.

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Table 2. Atomic Charges and Mulliken Spin Densities for the Electronic Ground State; also Included Are the Charges on the Histidine and Cysteine Fragments

	charge	spin density
Cu	0.42	34.8%
Nδ (His 46)	−0.62	1.7%
Nϵ (His 46)	−0.72	−0.004%
Sγ (Cys 112)	0.19	59.1%
Cβ (Cys 112)	0.68	−0.5%
Nδ (His 117)	−0.63	2.1%
Nϵ (His 117)	−0.71	0.004%
His 46	0.18	
Cys 112	0.19	
His 117	0.21	

Table 3. Calculated and Experimental Transition Energies (cm^{-1}) and Oscillator Strengths for Azurin[a]

excitation	MRD-CI	experimental[b]	CNDO/S[c]
0 → 1	4800 (0.000)	5800[d]	5100 (0.003)
0 → 2	14400 (0.026)	10200[d]	13800 (0.186)
0 → 3	15400 (0.000)	13000 (0.007)[e]	15900 (0.038)
0 → 4	17000 (0.002)	15900 (0.05)[e]	18000 (0.003)
0 → 5	17100 (0.116)	17650[f]	19400 (0.003)
0 → 6	20700 (0.004)	20800 (0.002)[e]	22700 (0.000)
0 → 7	36800 (0.000)		23100 (0.000)
0 → 8	37000 (0.001)		25500 (0.002)

[a] The labels in the first column only concern our calculation and refer to the ground state 0 and the excited states 1−8. The third column contains experimental data, and the fourth, the results of a CNDO/S calculation. [b] From refs 11,30. [c] From ref 6. [d] From the circular dichroism spectrum. [e] From the absorption spectrum. [f] From a Gaussian deconvolution of the most intense band in the absorption spectrum.

The singly occupied molecular orbitals (SOMO) of the reference configurations for which the square of the coefficient in the expansion is larger than 0.2 are specified. Each SOMO is represented by the Mulliken densities of the atomic orbitals, calculated according to eq 1. The SOMOs of the ground state and the first excited state are largely delocalized over copper and sulfur. The SOMO of the ground state is best described as a copper−sulfur π antibonding orbital. Also, a slight amount of density is present at the histidines, and the interaction with copper is of σ antibonding character. The SOMO of the first excited state is σ antibonding between copper and sulfur. States 2−4 are characterized by SOMOs localized at copper. In states 5 and 6, the SOMO is again delocalized over copper and sulfur, and these orbitals can be considered the π- and σ-bonding complements of those in states 0 and 1, respectively. The SOMO of state 5 is delocalized for about 45% over histidine 117. The density on this histidine is of π character. The SOMOs of states 7 and 8 are the π orbitals of the respective histidines. State 9 comprises four configurations with about equal coefficients that have largely different SOMOs. The dominant configuration is identical to the one that appears in state 8.

Mulliken charges and spin densities, derived from the density matrixes of the CI wave functions, at the atoms in the ground state are given in Table 2. The charge on copper is about +0.5, the Mulliken density of the copper 3d shell is 9.5 and of the 4s shell 1.0. The partial charge is significantly less than implied by the valence state commonly indicated for copper (+2) in the oxidized protein. The nitrogens are all negatively charged (∼−0.7) and the Sγ is about neutral. The spin densities are large at copper (35%) and sulfur (59%). A small amount of spin density, about 2%, is present at each of the copper-coordinated δ nitrogens of the histidines.

The frequencies calculated for the electronic transitions from the ground state to states 1−8 are given in Table 3, along with the oscillator strengths. Two transitions with significant intensity have been found, of which the transition to the fifth excited state at 17100 cm^{-1} has largest oscillator strength.

The principal values and the directions of the principal axes (*xyz*) of the g tensor with respect to the reference axes XYZ are summarized in Table 4. A g_{zz} value of 2.214 is found. The rhombicity (i.e., $g_{yy} - g_{xx}$) of the tensor is 0.026. The calculated direction of the z axis is almost parallel to the z axis of the reference system and makes an angle of 84° with the plane spanned by copper and the two δ nitrogens of the histidines and an angle of about 3° with the direction found experimentally.

The anisotropic hyperfine tensors of the copper-coordinated δ nitrogens and the remote ϵ nitrogens of the histidines are given

in Table 5. The principal values for the ϵ nitrogens are smaller than those for the δ nitrogens. For the δ nitrogens, the calculated tensors are nearly axial, i.e., $A_{y''y''} \approx A_{z''z''} \approx -^{1}/_{2} A_{x''x''}$ and the principal values of the tensor for histidine 117 are about 1.3 times larger than those for histidine 46. Because of the near-axiality, only the directions of the x'' axes are given, which are almost parallel to the respective Cu−Nδ bond directions. For the ϵ nitrogens, the tensors are rhombic, i.e., $A_{y''y''} \neq A_{z''z''}$. Like those for the δ nitrogens, the x'' axes point approximately along the respective Cu−Nϵ directions.

Discussion

We discuss the **g** tensor with regard to the relative densities of the copper and sulfur orbitals in the SOMO (section A), the hyperfine tensors of the nitrogens of the histidines (section B) and the electronic spectrum (section C).

A. The g Tensor. The deviation of the principal values of the **g** tensor from the free electron value g_e is predominantly determined by spin−orbit coupling which is large at the heavy atoms copper and sulfur. Let us, for the moment, consider a largely simplified approach in order to obtain insight into the origin of the large deviation of g_{zz} from g_e. Because the first excited state is much closer in energy to the ground state than the others (cf. Table 3), a significant contribution to g_{zz} arises from spin−orbit-coupling (cf. eq 3) between the ground state and the first excited state. When the SOMOs in this two-state model are approximated by

$$\Psi_0^{(0)} = c_y^S p_y^S + c_{xy}^{Cu} d_{xy}^{Cu}$$
$$\Psi_1^{(0)} = c_x^S p_x^S - c_{x^2-y^2}^{Cu} d_{x^2-y^2}^{Cu} \quad (6)$$

where the coefficients of the copper and sulfur orbitals are real and positive (note that sulfur is on the negative X axis) and the overlap between the copper and sulfur orbitals is neglected, the expression for g_{zz} becomes

$$g_{zz} = 2.0023 +$$
$$2 \frac{(382 c_x^S c_y^S - 2*829 c_{xy}^{Cu} c_{x^2-y^2}^{Cu})(c_x^S c_y^S - 2 c_{xy}^{Cu} c_{x^2-y^2}^{Cu})}{E_1 - E_0} \quad (7)$$

In this equation, spin−orbit-coupling constants of 829 and 382 cm^{-1} are used for copper and sulfur.[25] Owing to the negative signs in eq 7, the contributions of copper and sulfur partially cancel. As a result, the correction to g_e is negligible for small

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Table 4. Calculated and Experimental **g** Tensors for Azurin[a]

| | g values | | | | principal axes | | | | | |
| | calcd | exptl | | calcd | | | | exptl | | |
				X	Y	Z		X	Y	Z
g_{xx}	2.044	2.0393	x	0.1362	0.9898	−0.0425	x	0.3900	0.9195	−0.0483
g_{yy}	2.070	2.0568	y	−0.9717	0.1418	0.1887	y	−0.9109	0.3929	0.1259
g_{zz}	2.214	2.273	z	0.1928	0.0156	0.9811	z	0.1346	−0.0050	0.9921

angles	calcd	exptl			calcd	exptl
∠ z, Cu−Nδ(His 46)	85°	90°	∠ x, Cu−Nδ(His 46)		33°	19°
∠ z, Cu−Nδ(His 117)	89°	89°	∠ y, Cu−Nδ(His 117)		129°	145°
∠ z, CuNδNδ plane	84°	89°				

[a] The experimental tensor has been reproduced from ref 14. The directions of the principal axes of the **g** tensor are specified in the XYZ axes system (cf. Methods). Also included are the angles (deg) between the directions of the principal axes and a number of copper-ligand directions.

Table 5. Calculated and Experimental Principal Values [MHz] and Principal Directions of the Anisotropic Hyperfine Tensors for the Nδ and Nε Atoms of the Copper-Coordinating Histidines in Azurin[a]

| | | principal values | | | principal axes | | | | | | |
| | | calcd | exptl | | calcd | | | | exptl | | |
					X	Y	Z		X	Y	Z
							His 46				
Nδ											
	$A_{x''x''}$	1.90	1.0 (1.5)	x''	−0.6572	−0.7531	0.0301	x''	−0.6946	−0.7189	0.0221
	$A_{y''y''}$	−0.90	−0.1 (−0.5)					y''	0.6915	−0.6582	0.2987
	$A_{z''z''}$	−1.00	−0.9 (−1.0)					z''	−0.1992	0.2227	0.9558
Nε											
	$A_{x''x''}$	0.14	0.23	x''	0.8216	0.5692	0.0299	x''	0.8228	0.5690	0.0234
	$A_{y''y''}$	−0.04	−0.07	y''	0.5419	−0.7963	0.2686	y''	0.5190	−0.7657	0.3792
	$A_{z''z''}$	−0.10	−0.18	z''	0.1768	−0.2045	−0.9628	z''	0.2336	−0.2999	−0.9265
							His 117				
Nδ											
	$A_{x''x''}$	2.27	2.7	x''	−0.5089	0.8539	0.1087	x''	−0.5161	0.8475	0.1260
	$A_{y''y''}$	−1.04	−1.1					y''	0.5498	0.4407	−0.7111
	$A_{z''z''}$	−1.22	−1.5					z''	−0.6575	−0.2970	−0.6942
Nε											
	$A_{x''x''}$	0.16	0.35	x''	0.6291	−0.7307	−0.2652	x''	0.6509	−0.7573	−0.0520
	$A_{y''y''}$	−0.06	−0.04	y''	0.4665	0.6278	−0.6231	y''	0.4115	0.4102	−0.8147
	$A_{z''z''}$	−0.10	−0.32	z''	0.6218	0.2683	0.7358	z''	0.6377	0.5092	0.5797

[a] The experimental hyperfine tensors for the Nδ's have been reproduced from ref 15, those for the Nε's from ref 16.

coefficients in front of the copper orbitals, while it may become even more than 1 for complete localization at copper. This consideration indicates that a substantial density at copper is necessary to reproduce the value of g_{zz}. For plastocyanin, a spin density of about 40% on copper was needed to reproduce the experimental g_{zz} value of 2.22, and the wave function was adjusted to achieve this distribution.[3] In the present calculation on the model site of azurin a Mulliken spin density at copper in the ground state of 35% (cf. Table 2) is found and a g_{zz} value of 2.214, close to the experimental value (cf. Table 4). The matrix element between the first excited state and the ground-state adds 0.122 to the free-electron g value, that of the sixth excited state and the ground-state adds another 0.065. The contribution of the other excited states is small.

Our initial attempts to calculate the **g** tensor using the MRD-CI method but without the effective core potential at copper failed. At best, spin density at copper of 22% and a g_{zz} value of 2.099 were obtained. These calculations made use of the basis set for copper according to Wachters[26] with 6;2;1;1;1;1, 5;1;1;2 and 3;2;1 contractions for s-, p-, and d-type functions, respectively (as in ref 27, Table 29.15.1) and an additional p function (which is a contraction of two Gaussians with exponents

0.155065 and 0.046199 and coefficients 0.46358 and 0.64375, respectively) and d function (with exponent 0.15) were added. In this calculation the unpaired electron is largely localized at sulfur for the ground and first excited states and at copper for the second up to the sixth state. This localization is already present at the SCF level, not unexpectedly and noted previously.[6] The localization at sulfur of the SOMO in the ground state did not essentially change when slight changes in the structure of the model site were considered. A 0.05 Å increase or decrease of the distance between copper and sulfur (by translation of the cysteine fragment along the X axis) was found to change the spin-density distribution in the calculation without the ECP by only 1%. For four model sites that represent the azurin molecules in the asymmetric unit of the unit cell, the spin density at copper was found to vary between 17 and 22%. The charges at the atoms hardly differ from those calculated with the effective core potential at copper. Only the copper 3d density becomes 9.8 at the cost of a decrease of the 4s density.

In addition, we have calculated the electric field in the protein by placing point charges on the atoms of the amino acids with partial charges according to GROMOS96.[28] The difference in

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(27) Poirer, R.; Kari, R.; Csizmadia, I. G. In *Handbook of Gaussian Basis Sets*; Elsevier Science Publishers: New York, 1985.

magnitude of the electric field at the position of the copper and the sulfur was found to be about 1300 cm,$^{-1}$ which is comparable to the effect observed for plastocyanin with the CASPT2 method[7] when the electric field was included in the calculation. When the electric field is included in our MRD-CI calculation without ECP, the Mulliken spin density at copper in the SOMO increases to 29%, and the g_{zz} value becomes 2.13. This value, although larger than that found without inclusion of the electric field, is still not in agreement with the experimental value of g_{zz}. Apparently, slight variations in the structure of the model site or inclusion of the protein environment do influence the relative spin densities on copper and sulfur but not to the extent that the g_{zz} value is reproduced.

In previous CASSCF calculations on models of plastocyanin,[7] Darwin and mass-velocity corrections were added using first-order perturbation theory. Small energy corrections on the order of 400 cm^{-1} were obtained. A treatment based on first-order perturbation theory does not allow the wave function to respond to relativistic corrections. We performed two closed-shell SCF calculations on the Cu$^+$ ion in which we treated the copper nonrelativistically and relativistically, in the latter case making use of the ECP. The upward shift in energy of the levels of the 3d electrons was found to be about 1700 cm^{-1}, significant compared to the energy mismatch of the copper and sulfur orbitals in the calculation without ECP for the model site of azurin. For a proper description of the electronic structure of the blue-copper site, the inclusion of relativistic effects seems to be important.

The **g** tensor has proven to allow a critical test of the calculated wave function of the SOMO of a blue-copper site. For the model site of azurin, the MRD-CI calculation which includes an effective core potential leads to a g_{zz} value of 2.214 and g_{xx} and g_{yy} values of 2.044 and 2.070, respectively, close to the experimental values (cf. Table 4). The direction of the principal z axis of the **g** tensor differs only 3°, that of the x and y axes about 15° from the experimental ones. The difference between g_{xx} and g_{yy} (i.e., the rhombicity) largely stems from the part of the wave function at the sulfur, in agreement with earlier observations for plastocyanin.[3] When the spin−orbit-coupling parameter at sulfur is artificially set to zero, g_{xx} and g_{yy} become 2.043 and 2.052, respectively.

B. The Nitrogen Hyperfine Tensors. The hyperfine tensors of the nitrogens at the histidines reflect the delocalization of the SOMO over the copper site. Not only the spin density on the histidines themselves determines the nitrogen hyperfine interactions but the large spin densities at copper and sulfur contribute as well. The principal values $A_{x''x''}$, $A_{y''y''}$ and $A_{z''z''}$ are about 1 order of magnitude larger for the copper-coordinated δ nitrogens than for the remote ϵ nitrogens (cf. Table 5). This difference results from the fact that the ϵ nitrogens both are further away from copper and sulfur and carry virtually zero spin density (cf. Table 2). The anisotropic hyperfine interaction at the Nδs is about axial ($A_{y''y''} \approx A_{z''z''}$). The anistropic hyperfine interaction at the Nϵs is rhombic, which implies that the contribution of the spin density at copper is not dominant, as it would lead to an axial tensor.

From 95 GHz electron nuclear double resonance (ENDOR) and electron spin−echo envelope modulation (ESEEM) spec-

troscopy on a single crystal of azurin, the hyperfine tensors of the imidazole nitrogens of the copper-coordinated histidines have been determined experimentally.[15,16] The anisotropic parts are taken from refs 16 and 15, rotated to the axes system XYZ and included in Table 5. Agreement between calculation and experiment is satisfactory. The experimental principal values for the Nδ of histidine 46 are not known accurately, and two sets of principal values are specified[15] (cf. Table 5). The calculated principal values agree reasonably well with the second set (denoted in Table 5 between brackets), albeit that this set is slightly more rhombic. For the Nδ of histidine 117 the calculated principal values approach the experimental ones and the directions of the principal x'' axes agree within 2°. In line with the calculation, the experimental tensors of the ϵ nitrogens are not axial. The principal values are about a factor of 2 larger than the calculated ones. This difference may seem large but the spin density on the Nϵ's themselves is extremely small. A minor increase of this local contribution will have an appreciable effect on the hyperfine tensor.

C. The Electronic Spectrum. In this section we discuss the MRD-CI calculation with respect to the electronic spectrum and the charge redistribution upon excitation. Two electronic transitions are found with appreciable oscillator strength. These concern transitions to the second and the fifth excited state and calculated at 14400 and 17100 cm^{-1} (cf. Table 3). In the former transition, an electron is promoted from an orbital localized at copper (cf. Table 1, state 2) to the SOMO of the ground state which is (Cu d$_{xy}$ − S p$_y$) π antibonding (cf. state 0). In the latter transition an electron is promoted from an orbital which is comprised of a copper d$_{xy}$ orbital, a sulfur p$_y$ orbital that is π bonding to the d$_{xy}$ orbital, and an orbital on histidine 117 (cf. state 5) to the SOMO of the ground state. In state 0, the small density at the histidines mainly concerns σ orbitals at the Nδ's that are antibonding to the d$_{xy}$ orbital, while in state 5, the part of the SOMO at the histidine is of π character. Note the asymmetry as regards the admixture of the π orbitals at histidines 46 and 117. Particularly, the π orbital on histidine 117 contributes. This does not change when the distances of the coordinated nitrogens to the copper were taken equal.[16] The asymmetry is probably related to the relative orientation of the respective imidazole planes. The angle between the plane of the imidazole and the XY plane amounts to 17° and 45° degrees for the 46 and 117 position, respectively. The near coincidence of the XY plane and the plane of the imidazole of histidine 46 leads to a negligible overlap of the π orbital with the d$_{xy}$ orbital at copper and limits the delocalization of the molecular orbital over this histidine.

Given the π bonding and antibonding character between copper and sulfur of the SOMO of states 5 and 0, respectively, the most intense transition that causes the blue color of the protein is best referred to as a $\pi \rightarrow \pi^*$ transition. Previously, the transition was called a ligand-to-metal charge-transfer transition.[4] Our MRD-CI calculation reveals that the copper charges in states 0 and 5 are 28.6 and 28.4 and the sulfur charges are 15.8 and 16.0, and negligible charge is transferred.

For comparison, the electronic transitions observed for azurin[11,29] and calculated with the CNDO/S method[6] are included in Table 3. Both calculations yield two transitions with

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(29) Horio, T. *J. Biochemistry* **1958**, *45*, 195−205.

appreciable oscillator strength. The experimental spectrum is dominated by an intense band with a maximum at 15900 cm^{-1} (630 nm) that shows a shoulder of significant intensity at about 13000 cm^{-1} (770 nm). The result of the MRD-CI calculation leads us to identify the intense band with the transition calculated at 17100 cm^{-1} and the shoulder with the transition calculated at 14400 cm^{-1}. The difference between the calculated and the observed frequency for the band with maximum oscillator strength is about 1200 cm^{-1}. This difference is small in view of the width of the band in the experimental spectrum, the fact that the calculation concerns a copper site in a vacuum and the observation that the calculated frequency of the corresponding band for models of plastocyanin is found to be sensitive to slight changes in the Cu−S(Cys) bond length (∼2000 cm^{-1} per 0.05 Å[7]). The near-infrared and visible absorption spectrum of plastocyanin resembles that of azurin. Quantum-chemical calculations on model sites of plastocyanin have provided assignments of the transitions compatible to those obtained here for azurin.[4,7] However, the most advanced calculations as yet for plastocyanin have failed to reproduce the oscillator strength of the transition with the largest but one intensity.[7]

Concluding Remarks

Quantum-chemical studies on blue-copper proteins up to now mostly concerned the calculation of the optical transitions. In the present ab initio MRD-CI investigations on a model site of azurin the emphasis was on the EPR parameters, which allowed a critical test of the wave function of the ground state. The singly occupied molecular orbital in this wave function is best described as a linear combination of a d_{xy} orbital at copper that is π antibonding to a p_y orbital at sulfur and σ antibonding to the lone-pair orbitals at the δ nitrogens of the histidines. To properly describe the delocalization of the unpaired electron over copper and sulfur, an effective core potential including relativistic effects at copper had to be used. In that case, a Mulliken spin density distribution over copper and sulfur of 35% and 59% has been found, which reproduces the experimental **g** tensor. About 4% spin density is located at the histidines. The

agreement between the calculated and experimental hyperfine tensors of the copper-coordinated nitrogens reveals that the calculated wave function essentially describes the delocalization of the SOMO over the histidines. As yet, size limitations in the configuration-interaction calculation compelled us to study a truncated model of azurin that included the three strong equatorial ligands of copper. Structural and spectroscopic data reveal that the copper forms weak bonds with two axial ligands and that the cysteine Sγ and the histidine Nϵ hydrogens are involved in hydrogen bonds. Future ab initio calculations on larger sites have to address the role of these interactions as regards the delocalization of the ground-state wave function.

Note Added in Proof. After submission, a paper by A.R. Jaszewski and J. Jezierska has appeared (Chem. Phys. Lett. **2001**, *343*, 571− 580) concerning the calculation of hyperfine parameters for azurin by hybrid density functional theory.

Supporting Information Available: Cartesian coordinates of the atoms comprising the model of the blue-copper site of *P. aeruginosa* azurin (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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