

Quantum chemical calculations of the reorganization energy of blue-copper proteins

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Abstract

The inner-sphere reorganization energy for several copper complexes related to the active site in blue-copper protein has been calculated with the density functional B3LYP method. The best model of the blue-copper proteins, $\text{Cu}(\text{Im})_2(\text{SCH}_3)(\text{S}(\text{CH}_3)_2)^{0/+}$, has a self-exchange inner-sphere reorganization energy of 62 kJ/mol, which is at least 120 kJ/mol lower than for $\text{Cu}(\text{H}_2\text{O})_4^{+2+}$. This lowering of the reorganization energy is caused by the soft ligands in the blue-copper site, especially the cysteine thiolate and the methionine thioether groups. Soft ligands both make the potential surfaces of the complexes flatter and give rise to oxidized structures that are quite close to a tetrahedron (rather than tetragonal). Approximately half of the reorganization energy originates from changes in the copper-ligand bond lengths and half of this contribution comes from the Cu-S_{Cys} bond. A tetragonal site, which is present in the rhombic type 1 blue-copper proteins, has a slightly higher (16 kJ/mol) inner-sphere reorganization energy than a trigonal site, present in the axial type 1 copper proteins. A site with the methionine ligand replaced by an amide group, as in stercyanin, has an even higher reorganization energy, about 90 kJ/mol.

Keywords: B3LYP method; blue-copper proteins; entatic state theory; induced-rack theory; reorganization energy

Blue-copper proteins are a class of electron transfer proteins that differs from most small inorganic copper complexes in a number of properties. For example, they normally exhibit an intense blue color, an electron spin resonance spectrum with narrow hyperfine splittings, and an unusually high reduction potential (Sykes, 1990; Adman, 1991). Furthermore, their crystal structures show an unusual trigonal cupric geometry (Colman et al., 1978; Guss et al., 1986; Guss et al., 1992). The copper ion is coordinated by three strong ligands forming an approximate trigonal plane: one cysteine thiolate ion and two histidine nitrogen atoms. In addition, an axial ligand binds to the copper ion at a longer distance. In most proteins, the axial ligand is a methionine thioether group, but in stercyanin and related proteins, it is instead a glutamine amide group. In azurin both methionine and a backbone amide oxygen are axial ligands.

In the 1960s it was suggested that the anomalous properties of the blue-copper proteins are caused by an unnatural cupric coordination geometry. More precisely, the entatic state and the induced-rack hypotheses propose that the protein forces the Cu(II) ion to bind in a geometry similar to the one preferred by Cu(I), i.e., tetrahedral (Malmström, 1994; Williams, 1995). This strained ge-

ometry of the oxidized copper state would give rise to an energized state and a small change in coordination geometry during reduction, which would facilitate electron transfer.

However, these suggestions have recently been challenged by quantum chemical geometry optimizations, showing that a Cu(II) ion with the same ligands as in the protein assumes a geometry that is very similar to the one observed experimentally (Ryde et al., 1996). Further investigations have shown that the spectroscopic features as well as the trigonal structure can instead be traced back to the copper-cysteine interaction (Pierloot et al., 1997; Olsson et al., 1998). In the typical blue-copper proteins, copper and the cysteine thiolate group forms a highly covalent π bond where a 3p orbital from the sulfur atom overlaps with two lobes of a Cu 3d orbital. Thus, S_{Cys} formally occupies two positions in a square coordination and additional ligands have to coordinate as axial ligands above and below the trigonal plane. Yet, in another type of blue-copper protein, the rhombic type 1 proteins, the electronic structure is more similar to the one in small inorganic copper complexes, i.e., the copper ion forms σ bonds to four strong ligands in a tetragonal arrangement, including the cysteine thiolate group (the bond in this group is actually a mixture of σ and π interactions). Nevertheless, these structures are also close to tetrahedral. This is because the cysteine thiolate group is large and soft and forms a strongly covalent bond to the copper ion, in which charge is transferred from the thiolate group to the Cu(II) ion (Olsson et al., 1998).

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The blue-copper proteins are electron transfer agents. According to the Marcus theory (Marcus & Sutin, 1985), the rate of electron transfer is given by

$$k_{ET} = \frac{2\pi}{\hbar} \frac{H_{DA}^2}{\sqrt{4\pi\lambda RT}} \exp\left(\frac{-(\Delta G^0 + \lambda)^2}{4\lambda RT}\right). \quad (1)$$

Here, H_{DA} is the electronic coupling matrix element, which depends on the overlap of the wavefunctions of the two states involved in the reaction, ΔG^0 is the free energy of the reaction, i.e., the reduction potential, and λ is the reorganization energy, the energy associated with relaxing the geometry of the system after electron transfer (Fig. 1). If the structures before and after the reaction are similar, the reorganization energy will be small, and electron transfer will be fast. Thus, for electron transfer proteins it is of vital importance to reduce the reorganization energy and therefore the evolution process will favor proteins in which the redox-active metal ion assumes similar coordination geometries in the relevant oxidation states.

For convenience, the reorganization energy is usually divided into two parts: inner-sphere and outer-sphere reorganization energy, depending on which atoms are relaxed. For a metal-containing protein, the inner-sphere reorganization energy is associated with the structural change of the first coordination sphere, whereas the outer-sphere reorganization energy involves the structural change of the remaining protein as well as the solvent.

In this paper, we calculate the reorganization energy of blue-copper proteins using quantum chemical methods. We have concentrated on the inner-sphere reorganization energy, since we believe that it is most important for the understanding of the evolutionary design of the proteins. Moreover, the inner-sphere reorganization energies can be predicted to vary less between various proteins than the total reorganization energy, since the solvent exposure of the complexes (especially when the protein is docked with the corresponding acceptor or donor) varies more than the copper coordination geometry. By comparing the reorganization energy of a number of models related to the active copper site, we estimate the role of the four ligands for the low reorganization energy in

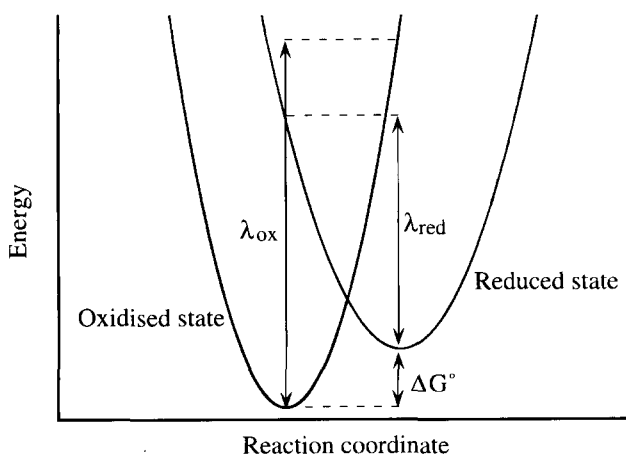


Fig. 1. The potential energy of a general electron-transfer reaction as a function of an (ill-defined) reaction coordinate. The energy barrier of the reaction ΔG^0 and the reorganization energy of the reduction and the oxidation (λ_{red} and λ_{ox}) are indicated.

these proteins. Furthermore, by comparing models with different axial ligands and electronic structures, we examine the difference between various types of blue-copper proteins.

Results and discussion

Inner-sphere reorganization energy

One goal of this investigation was to estimate the reorganization energy for a number of small copper complexes and to determine the influence of different ligands on the inner-sphere reorganization. The geometries of all optimized complexes are described in Tables 1 and 2, and the calculated reorganization energies are collected in Table 3.

A natural start of such an investigation is to study a copper ion in aqueous solution. Therefore, we started with octahedral $\text{Cu}(\text{H}_2\text{O})_6^{+2}$ complexes. For Cu(II), the optimal structure is six-coordinate as expected. However, for Cu(I), the equilibrium structure had only three direct ligands, with the other three ligands bound to the first sphere ligands in an intricate hydrogen bond network. The self-exchange inner-sphere reorganization energy ($\lambda_i = \lambda_{ox} + \lambda_{red}$) for these two structures is high, 336 kJ/mol. The major part of this energy is due to the change in the coordination number. A six-coordinate $\text{Cu}(\text{H}_2\text{O})_6^+$ complex could be obtained if the system was forced to have D_{2h} symmetry. This complex had an appreciably lower reorganization energy, 112 kJ/mol. It is notable that λ_{red} is much lower than λ_{ox} for this complex (36 compared to 76 kJ/mol). This is simply because the force constants for the equatorial Cu(II)-O bonds are more than three times as large as those of the Cu(I)-O bonds.

In most proteins, the copper ion has four rather than six ligands. Therefore, we next calculated the reorganization energy for $\text{Cu}(\text{H}_2\text{O})_4^{+2}$. Also in this complex, the optimal reduced structure is three-coordinate. This three-coordinate structure had a higher reorganization energy than a structure forced to be four-coordinate, 247 kJ/mol compared to 186 kJ/mol. Again, λ_{ox} is about twice as large as λ_{red} . The larger reorganization energy compared to the (constrained) six-coordinate system can be rationalized considering the structure. Both the oxidized and reduced forms of the six-coordinate complex are (distorted) octahedral. The only significant change when the complex is reduced is an elongation of the four equatorial Cu-O bonds. However, in the four-coordinate complexes, the situation is quite different. Due to Jahn-Teller effects, $\text{Cu}(\text{H}_2\text{O})_4^{+2}$ is square-planar, whereas the four-coordinate reduced complex is tetrahedral. Thus, the geometry of the two complexes is quite different and the rearrangement of the ligands between the equilibrium structures will involve a sizable expenditure of energy. However, the most interesting conclusion of this calculation is that a lowering of the coordination number of the copper ion from six to four is unfavorable for the reorganization energy. This lowering is necessary since Cu(I) normally have a coordination number lower than six.

In proteins, the most common ligand is histidine. Therefore, we next studied a four-coordinate complex with only nitrogen donor atoms. For simplicity, we used ammonia instead of the more realistic imidazole ligand. $\text{Cu}(\text{NH}_3)_4^{2+}$ is square-planar with a slight distortion toward a tetrahedron, whereas $\text{Cu}(\text{NH}_3)_4^+$ is four-coordinate and tetrahedral. Interestingly, $\text{Cu}(\text{NH}_3)_4^{+2}$ has a much lower reorganization energy than $\text{Cu}(\text{H}_2\text{O})_4^{+2}$ (135 kJ/mol). This lower reorganization energy is probably due to the smaller force constants (1.7 to 2.5 times smaller), the smaller

Table 1. Bond distances (pm) and the number of imaginary frequencies of the investigated complexes, together with a specification of the constraints used for some complexes^a

Bond (Å)	L ₁	L ₂	L ₃	L ₄	Imaginary frequency ^b
Cu(H ₂ O) ₆ ²⁺	202 202	202 202	230	230	0
Cu(H ₂ O) ₆ ⁺	197 198	231 307	319	357	0
Cu(H ₂ O) ₆ ^c	226 226	227 227	227	227	8
Cu(H ₂ O) ₄ ²⁺	197	197	197	197	0
Cu(H ₂ O) ₄ ⁺	191	195	197	256	0
Cu(H ₂ O) ₄ ^d	214 ^e	214 ^e	214 ^e	214 ^e	1
Cu(NH ₃) ₄ ²⁺	206	206	206	206	0
Cu(NH ₃) ₄ ⁺	215	215	215	215	0
Cu(NH ₃) ₃ (SH ₂) ²⁺	248	203	204	208	0
Cu(NH ₃) ₃ (SH ₂) ⁺	244	212	212	214	0
Cu(NH ₃) ₃ (SH) ⁺	224	208	210	212	0
Cu(NH ₃) ₃ (SH)	226	204	217	347	0
Cu(NH ₃) ₃ (SH) ^d	235	217 ^e	217 ^e	217 ^e	2
Cu(NH ₃) ₂ (SH)(SH ₂) ⁺ tetragonal	225	205	207	254	0
Cu(NH ₃) ₂ (SH)(SH ₂) ⁺ trigonal	217	207	207	284	0
Cu(NH ₃) ₂ (SH)(SH ₂)	231	221	217	232	0
Cu(NH ₃) ₂ SH(NH ₂ HCO) ⁺	216	206	206	227	0
Cu(NH ₃) ₂ SH(NH ₂ HCO)	220	212	213	330	0
Cu(NH ₃) ₂ SH(NH ₂ HCO) ^d	224	214	213	240 ^e	0
Cu(Im) ₂ (SCH ₃)(S(CH ₃) ₂) ⁺ trigonal	218	204	205	267	
Cu(Im) ₂ (SCH ₃)(S(CH ₃) ₂)	232	214	215	237	
Cu(Im) ₂ (SCH ₃)(S(CH ₃) ₂) ^d	227	205	210	290 ^e	
Cu(Im) ₂ (SCH ₃)(S(CH ₃) ₂) ^f	207	191	206	282	
Cu(Im) ₂ (SCH ₃)(S(CH ₃) ₂) ^f	217	212	239	287	
Cu(Im) ₂ (SH)(S(CH ₃) ₂) ⁺ tetragonal	223	205	206	242	
Cu(Im) ₂ (SH)(S(CH ₃) ₂)	233	214	214	237	
Cu(Im) ₂ (SH)(S(CH ₃) ₂) ^d	229	205	210	290 ^e	
Cu(Im) ₂ (SCH ₃)(CH ₃ CONH ₂) ⁺	217	202	206	224	
Cu(Im) ₂ (SCH ₃)(CH ₃ CONH ₂)	221	210	204	362	
Cu(Im) ₂ (SCH ₃)(CH ₃ CONH ₂) ^d	226	208	209	240 ^e	

^aIf the ligands are the same, they are ordered by their bond lengths. If three ligands are the same, L₁ is the hetero-ligand. For the blue-copper models, L₁ is the cysteine model and L₄ is the model of the axial ligand.

^bFor the structures obtained without any constraints, no imaginary frequencies indicate that the structure is a stable equilibrium state (a transition state has one imaginary frequency). For the constrained structures, the number of imaginary frequencies reflects the curvature of the potential surface around the constrained geometry.

^cThe structure was constrained to have D_{2h} symmetry.

^dThe structure involves some bond-length constraints.

^eThis distance has been constrained.

^fCrystal structure (Guss et al., 1986, 1992).

difference in the Cu-ligand bond lengths (Table 1), and the fact that ammonia bound to a copper ion cannot form hydrogen bonds (which water can). Thus, by simply changing the ligand atoms from oxygen to nitrogen, the complexes regain almost all the energy lost when the coordination number was reduced from six to four.

The typical blue-copper site consists of one cysteine, one methionine, and two histidine ligands. Thus, we next replaced one of the ammonia ligands in Cu(NH₃)₄⁺²⁺ by either SH₂ or SH⁻ (simple models of methionine and cysteine, respectively). The structures of the two oxidation states of the Cu(NH₃)₃(SH₂)⁺²⁺ complex are very similar to those of the corresponding Cu(NH₃)₄⁺²⁺ complex, except for the longer Cu-S bond. However, the reorganization energy is 14 kJ/mol lower than for Cu(NH₃)₄⁺²⁺ (λ_i = 122 kJ/mol). This is an effect of the much softer Cu-S bond (the force constant is 0.009 kJ/mol/pm², compared to 0.021 kJ/mol/pm² for Cu-N).

The structures of the Cu(NH₃)₃(SH)^{0/+} complexes differ more from those of the Cu(NH₃)₄⁺²⁺ complexes. Cu(NH₃)₃(SH)⁺ is still tetragonal, but much more distorted toward a tetrahedron than the corresponding NH₃ or SH₂ complexes. The optimal structure of the reduced complex is three-coordinate. This is due to the negatively charged SH⁻ ligand, which is an excellent hydrogen bond acceptor and donates charge to the Cu(I) ion, thereby destabilizing bonds to other ligands. Therefore, it is more favorable for the third ammonia ligand to form a hydrogen bond to SH⁻ and another ammonia ligand than to bind to the copper ion. Interestingly, the reorganization energy of the three-coordinate complex is only 134 kJ/mol although the coordination number changes. This is almost the same energy as for Cu(NH₃)₄⁺²⁺. Moreover, we have also optimized a structure of the reduced complex, forced to be four-coordinate. Naturally, it has an even lower reorganization energy, 91 kJ/mol, 31 kJ/mol lower than the one of

Table 2. Bond angles (degrees) of the investigated complexes^a

Angle (deg)	(L ₁ -Cu-L ₂)	(L ₁ -Cu-L ₃)	(L ₁ -Cu-L ₄)	(L ₂ -Cu-L ₃)	(L ₂ -Cu-L ₄)	(L ₃ -Cu-L ₄)	Σθ ^b
Cu(H ₂ O) ₄ ²⁺	90	90	174	175	90	90	208
Cu(H ₂ O) ₄ ⁺ c	106	94	131	91	135	104	90
Cu(NH ₃) ₄ ²⁺	92	92	158	158	92	92	167
Cu(NH ₃) ₄ ⁺	109	109	110	109	110	110	3
Cu(NH ₃) ₃ (SH ₂) ²⁺	96	88	150	159	94	93	157
Cu(NH ₃) ₃ (SH ₂) ⁺	110	110	99	113	112	112	20
Cu(NH ₃) ₃ (SH) ⁺	99	91	143	138	98	98	114
Cu(NH ₃) ₃ (SH) ^c	101	101	112	118	112	112	33
Cu(NH ₃) ₂ (SH)(SH ₂) ⁺ tetragonal	100	93	136	141	101	94	108
Cu(NH ₃) ₂ (SH)(SH ₂) ⁺ trigonal	124	124	110	103	93	93	69
Cu(NH ₃) ₂ (SH)(SH ₂)	100	101	120	112	105	119	45
Cu(NH ₃) ₂ SH(NH ₂ HCO) ⁺	125	125	116	103	87	87	89
Cu(NH ₃) ₂ SH(NH ₂ HCO)	128	130	109	96	65	65	142
Cu(NH ₃) ₂ SH(NH ₂ HCO) ^c	123	130	112	106	82	81	96
Cu(Im) ₂ (SCH ₃)(S(CH ₃) ₂) ⁺ trigonal	120	122	116	103	95	94	66
Cu(Im) ₂ (SCH ₃)(S(CH ₃) ₂)	108	105	115	109	113	107	18
Cu(Im) ₂ (SCH ₃)(S(CH ₃) ₂) ^c	112	120	99	119	101	100	51
Cu(Im) ₂ (SCH ₃)(S(CH ₃) ₂) ^d	132	121	110	97	88	101	77
Cu(Im) ₂ (SCH ₃)(S(CH ₃) ₂) ^d	136	110	113	99	88	106	66
Cu(Im) ₂ (SH)(S(CH ₃) ₂) ⁺ tetragonal	141	97	103	100	95	126	91
Cu(Im) ₂ (SH)(S(CH ₃) ₂)	108	107	111	110	112	109	9
Cu(Im) ₂ (SH)(S(CH ₃) ₂) ^c	120	112	97	121	102	98	56
Cu(Im) ₂ (SCH ₃)(CH ₃ CONH ₂) ⁺	125	122	113	103	92	95	70
Cu(Im) ₂ (SCH ₃)(CH ₃ CONH ₂)	123	130	100	106	81	78	107
Cu(Im) ₂ (SCH ₃)(CH ₃ CONH ₂) ^c	122	123	111	111	89	88	71

^aIf the ligands are the same, they are ordered by their bond lengths. If three ligands are the same, L₁ is the hetero-ligand. For the blue-copper models, L₁ is the cysteine model and L₄ is the model of the axial ligand.

^bThe sum of the difference between the six angles and the ideal tetrahedral angle (109.47°).

^cThe structure has been constrained.

^dCrystal structure (Guss et al., 1986, 1992).

Cu(NH₃)₃(SH₂)⁺²⁺. Thus, the cysteine model gives a lower reorganization energy than both the histidine and methionine models.

Clearly, this is not due to the Cu-S force constant, since it is larger than for Cu-N. Instead, the reason seems to be that the oxidized structure is more tetrahedral than the other structures we have studied. This can be seen from the last column in Table 2, which gives the sum of the deviations of the angles in the complex from the ideal tetrahedral angles (Σθ). In Cu(H₂O)₄²⁺, Cu(NH₃)₄²⁺, and Cu(NH₃)₃(SH₂)²⁺, these sums are 157–208°, indicating structures close to square planar. However, in Cu(NH₃)₃(SH)⁺, Σθ = 114°, showing that the structure is much more close to a tetrahedron. The tetrahedral structure of the Cu(NH₃)₃(SH)⁺ complex is in its turn caused by the covalent copper-thiolate interaction, where the polarizable SH⁻ ion donates charge to the copper ion, making it more similar to a Cu(I) ion (Olsson et al., 1998).

The final step on our way toward the blue-copper site is to study complexes involving both a cysteine and a methionine model. The simplest such complex, Cu(NH₃)₂(SH)(SH₂)^{0/+}, has a reorganization energy of 75 kJ/mol. This is 61 kJ/mol lower than for Cu(NH₃)₄⁺²⁺ and 17 kJ/mol lower than for Cu(NH₃)₃(SH)^{0/+}. Thus, the concerted effect of the cysteine and methionine models is close to the sum of their individual effects. Again, the reason for the low reorganization energy of this complex is that the oxidized structure is rather close to tetrahedral; Σθ is 108°, 6° less than for Cu(NH₃)₃(SH)⁺.

Table 3. Reorganization energies of the investigated complexes (kJ/mol)

Model complex (kJ/mol)	λ _{red}	λ _{ox}	λ _i
Cu(H ₂ O) ₆ ⁺²⁺	152.9	182.8	335.7
Cu(H ₂ O) ₆ ^{+2+a}	35.9	76.2	112.1
Cu(H ₂ O) ₄ ⁺²⁺	85.8	160.9	246.7
Cu(H ₂ O) ₄ ^{+2+a}	60.8	124.8	185.6
Cu(NH ₃) ₄ ⁺²⁺	67.7	67.8	135.5
Cu(NH ₃) ₃ (SH ₂) ⁺²⁺	59.0	62.8	121.8
Cu(NH ₃) ₃ (SH) ^{0/+}	80.9	52.7	133.6
Cu(NH ₃) ₃ (SH) ^{0/+a}	43.7	47.6	91.3
Cu(NH ₃) ₂ (SH)(SH ₂) ^{0/+} tetragonal	39.8	34.7	74.5
Cu(NH ₃) ₂ (SH)(SH ₂) ^{0/+} trigonal	21.9	44.2	66.1
Cu(NH ₃) ₂ SH(NH ₂ HCO) ^{0/+} trigonal	86.8	78.6	165.4
Cu(NH ₃) ₂ SH(NH ₂ HCO) ^{0/+} trigonal ^a	70.3	45.6	115.9
Cu(Im) ₂ (SCH ₃)(S(CH ₃) ₂) ^{0/+} trigonal	32.7	28.8	61.5
Cu(Im) ₂ (SCH ₃)(S(CH ₃) ₂) ^{0/+} trigonal ^a	28.2	37.8	66.0
Cu(Im) ₂ (SH)(S(CH ₃) ₂) ^{0/+} tetragonal	37.7	39.8	77.5
Cu(Im) ₂ (SH)(S(CH ₃) ₂) ^{0/+} tetragonal ^a	33.0	48.6	81.6
Cu(Im) ₂ (SCH ₃)(CH ₃ CONH ₂) ^{0/+} trigonal	72.4	60.6	133.0
Cu(Im) ₂ (SCH ₃)(CH ₃ CONH ₂) ^{0/+} trigonal ^a	59.3	30.6	89.9

^aThe reduced structure has been constrained.

Interestingly, there are two stable geometries of the $\text{Cu}(\text{NH}_3)_2(\text{SH})(\text{SH}_2)^+$ complex, characterized by different electronic structures. The one we have considered up to now has the same structure as all the other Cu(II) complexes. In these, the copper ion forms σ bonds with the four equatorial ligands. However, in the other $\text{Cu}(\text{NH}_3)_2(\text{SH})(\text{SH}_2)^+$ structure, the two ammonia ligands still form σ bonds with two of the lobes of the singly-occupied Cu 3d orbital, but the thiolate group instead forms a π bond with the other two lobes of the Cu 3d orbital. Then, SH_2 cannot overlap with this orbital, and therefore it has to become an axial ligand that binds by pure electrostatic interactions. In other words, $\text{Cu}(\text{NH}_3)_2(\text{SH})(\text{SH}_2)^+$ has a *trigonal* structure, whereas all the other oxidized structures have been tetragonal (Olsson et al., 1998).

In fact, the trigonal structure of $\text{Cu}(\text{NH}_3)_2(\text{SH})(\text{SH}_2)^+$ is more stable than the tetragonal one (by 9 kJ/mol (Olsson et al., 1998)). The trigonal structure is even more similar to a tetrahedron than the tetragonal one, with $\Sigma\theta = 69^\circ$. Therefore, the reorganization energy is also lower, only 66 kJ/mol. It is especially λ_{red} that is low, 22 kJ/mol, whereas λ_{ox} is twice as large. Most interestingly, a comparison with protein crystal structures shows that the trigonal structure is a simple model for the normal type 1 blue-copper proteins, such as plastocyanin, whereas the tetragonal structure is a model of the rhombic type 1 proteins, such as nitrite reductase, pseudoazurin, and cucumber basic protein (Olsson et al., 1998; Pierloot et al., 1998).

To get more accurate estimates of the inner-sphere reorganization energy of the copper site in the blue-copper proteins, we have also performed calculations with more realistic ligand models. For the trigonal structure, we have studied the $\text{Cu}(\text{Im})_2(\text{SCH}_3)(\text{S}(\text{CH}_3)_2)^{0/+}$ complexes. The structures of these complexes are rather similar to those of the $\text{Cu}(\text{NH}_3)_2(\text{SH})(\text{SH}_2)^{0/+}$ models and they are also similar to the crystal structures of oxidized and reduced plastocyanin (Ryde et al., 1996). The only significant difference is that the Cu- S_{Met} bond in the reduced structure is shorter than in the crystal structures, 237 compared to 290 pm. The reorganization energy of $\text{Cu}(\text{Im})_2(\text{SCH}_3)(\text{S}(\text{CH}_3)_2)^{0/+}$ is 62 kJ/mol, which is close to the value found for $\text{Cu}(\text{NH}_3)_2(\text{SH})(\text{SH}_2)^{0/+}$. However, the difference between λ_{ox} and λ_{red} is smaller, only 4 kJ/mol. The reorganization energy does not change significantly if the Cu- S_{Met} bond length in the reduced structure is constrained to the experimental value (66 kJ/mol). This is because the potential surface of the Cu- S_{Met} bond is very flat. In fact, this bond length can be changed by over 50 pm around the minimum value at an expense of less than 5 kJ/mol (Ryde et al., 1996).

For the tetragonal structure present in the rhombic type 1 copper proteins, our best model is $\text{Cu}(\text{Im})_2(\text{SH})(\text{S}(\text{CH}_3)_2)^{0/+}$, since we have not been able to optimize such a structure for $\text{Cu}(\text{Im})_2(\text{SCH}_3)(\text{S}(\text{CH}_3)_2)^{0/+}$ (Pierloot et al., 1998). This complex gives a reorganization energy of 78 kJ/mol for the optimal structures, or 82 kJ/mol if the Cu- S_{Met} bond is constrained to the experimental value. Thus, the tetragonal structures seem to give a slightly larger reorganization energy than the trigonal ones (16 kJ/mol). Yet, this difference is so small that both types of structures are found in electron transfer proteins.

As was mentioned above, there exist blue-copper proteins in which the axial methionine ligand has been replaced by a glutamine amide ligand, e.g., stellacyanin and umecyanin. We have investigated the effect of this substitution by studying the $\text{Cu}(\text{Im})_2(\text{SCH}_3)(\text{CH}_3\text{CONH}_2)^{0/+}$ model. In conformity with the other protein models, this complex may also assume a trigonal as

well as a tetragonal structure, the energy of which are very similar (less than 7 kJ/mol difference) (De Kerpel et al., 1998). However, the crystal structure of cucumber stellacyanin clearly shows a trigonal copper coordination geometry, so we used the trigonal model in this investigation. Quite unexpectedly, the reorganization energy of this complex turned out to be rather high, 90 kJ/mol, appreciably higher than for the other protein models. The reason seems to be that the Cu-O bond is shorter and the force constant is larger than for the Cu- S_{Met} bond. It is also notable that the optimal structure of the $\text{Cu}(\text{Im})_2(\text{SCH}_3)(\text{CH}_3\text{CONH}_2)$ complex in a vacuum is three-coordinate. If this structure (and not a complex constrained to be four-coordinate) is used for the calculations of the reorganization energy, the result is even higher, 133 kJ/mol.

Comparison with experiments

Only a few experimental measurements of the reorganization energy of blue-copper proteins have been published. Farver et al. (1996) have estimated the total reorganization energy for azurin from several species and mutants to be 99 kJ/mol. On the other hand, Gray et al. have calculated a total reorganization energy of 68–77 kJ/mol for the same enzyme (Di Bilio et al., 1997; Winkler et al., 1997). The reason for the difference in these estimates is that they involve different electron donors (i.e., different reactions). Gray et al. use Ru(II) complexes bound to a His residue, whereas Farver et al. (1996) measure electron transfer from a cystine disulfide radical ion to the copper ion. It is likely that the latter donor gives a 20 kJ/mol larger reorganization energy than the $\text{Ru}^{2+/3+}$ couple, thereby explaining the difference between the two estimates of the reorganization energy for azurin (Di Bilio et al., 1997). Thus, the self-exchange reorganization energy for the blue-copper site in azurin has been estimated to be 79 kJ/mol (using a value of the reorganization energy for the ruthenium complex of 75 kJ/mol) (Di Bilio et al., 1997).

These estimates are total self-exchange reorganization energies. To compare these to our inner-sphere reorganization energies, an estimate of the outer-sphere reorganization energy is needed. This is more complicated to calculate, since a proper treatment of both the protein and the solvent is needed, including dynamics and electrostatic polarization. Moreover, it strongly depends on the conformation of the docking complex between plastocyanin and its donor or acceptor proteins, the geometry of which is not known (Soriano et al., 1997). The latter point also shows that it is highly questionable to use Marcus' combination rules (Marcus & Sutin, 1985) to calculate the reorganization energy from other reactions where it has been measured. This applies to all self-exchange reorganization energies that cannot be measured directly, e.g., the one for azurin.

Soriano et al. (1997) have calculated the outer-sphere reorganization energy for three tentative configurations of the docking complex between plastocyanin and its natural electron donor, cytochrome *f*. They solve the Poisson–Boltzmann equation considering the proteins and the solvent as dielectric media characterized by different dielectric constants and taking account only of the charged residues in the proteins. They obtain an outer-sphere reorganization energy of 42–54 kJ/mol for the three complexes, of which the lower value corresponds to the complex that the authors consider to be most realistic.

If we add 42 kJ/mol to our estimates of the self-exchange inner-sphere reorganization energy, we obtain 104 kJ/mol, keeping in mind that this is questionable and very approximate. This is

25 kJ/mol higher than the energy estimated for azurin (Di Bilio et al., 1997). However, it is most likely that azurin has a lower inner-sphere reorganization energy than plastocyanin. In azurin, the copper ion forms a trigonal bipyramidal structure with five ligands, whereas plastocyanin has four ligands in a trigonal pyramidal geometry. In conformity with our results for the four- and six-coordinate water complexes (Table 3), the four-coordinate structure should have a higher reorganization energy since it would exhibit larger differences in the angles upon reduction than the five-coordinate azurin site. This is also confirmed by experimental measurements of the reorganization energy for plastocyanin (Sigfridsson et al., 1996; Soriano et al., 1997). For example, Sigfridsson et al. (1996) estimate the reorganization energy of ruthenium-modified plastocyanin to about 100 kJ/mol. Following the procedure of Di Bilio et al. (1997) this would give a self-exchange reorganization energy for plastocyanin of 125 kJ/mol, 21 kJ/mol more than our estimate. It should also be noted that experimental estimates of the reorganization energy of the same donor-acceptor complex involve an appreciable uncertainty. For example, estimates of the reorganization energy of azurin measured by Farver et al. have varied between 99 and 134 kJ/mol (Farver & Pecht, 1994; Farver et al., 1996). In conclusion, our calculated inner-sphere reorganization energies are in agreement with experimental estimates, considering the accuracy of the two types of energies.

Recently, Loppnow and Fraga (1997) have estimated the reorganization energy for plastocyanin from three different species, analyzing resonance Raman intensities (Fraga et al., 1996). They obtain a reorganization energy of 18 kJ/mol from the specific vibrations and another 6 kJ/mol from the solvent. Clearly, these energies are much lower than our estimates. However, they do not measure the reorganization energy for electron transfer, but rather for the charge-transfer excitation corresponding to the intense blue line (around 600 nm) in the spectrum of plastocyanin. This excitation has been attributed to a transition between and the ground state orbital involving a Cu-S_{Cys} π antibond to the corresponding bonding orbital (Gewirth & Solomon, 1988; Larsson et al., 1995; Pierloot et al., 1997). Formally, the excitation involves the movement of an electron from S_{Cys} to Cu. However, the Cu-S_{Cys} bond is strongly covalent and much charge is transferred to S_{Cys} already

in the ground state. In fact, only about 0.2e is transferred in the excitation, according to theoretical calculations (Pierloot et al., 1997). Thus, this charge transfer excitation is very different from true electron transfer reactions involving plastocyanin, and there is therefore no reason to expect that these results should reflect the reorganization energy of the latter reactions.

Several other groups have tried to calculate the reorganization energy for transition metal complexes or proteins (Churg et al., 1983; Zhou & Khan, 1989; Yadav et al., 1991; Bu et al., 1994; Larsson et al., 1995; Bu et al., 1997; Soriano et al., 1997). In particular, Larsson et al. (1995) have tried to estimate the inner-sphere reorganization energy for azurin and plastocyanin using crystal structures and simple harmonic potential for the metal ligand bonds. Unfortunately, uncertainties in the structures and the force constants make such an approach very unreliable, and the results ranged from 21 to 81 kJ/mol depending on which crystal structure was used.

Contributions to the inner-sphere reorganization energy

It is interesting to determine the contributions to the inner-sphere reorganization energy from the various internal coordinates of the investigated complexes, both to compare with experiment and to investigate whether the reorganization energy can be calculated by classical methods from force constants and geometry changes, as has been done in earlier work (Zhou & Khan, 1989; Larsson et al., 1995). Tables 4 and 5 show the results from such an investigation for the small model of plastocyanin, Cu(NH₃)₂(SH)(SH₂)^{0/+}. The partitioning is based on Equation 2 (see below), using the change in geometry between the oxidized and reduced complex combined by the harmonic force constants obtained from the Hessian matrix of the two complexes. Several points are noteworthy in this analysis.

The reorganization energy from the bond lengths in the reduced complex is strongly dominated by the contribution from the Cu-S_{Met} bond, 25 kJ/mol. This is clearly an overestimate. According to the crystal structures of the blue-copper proteins, this bond does not decrease very much on reduction (only about 7 pm) (Guss et al., 1986, 1992; Ryde et al., 1996). As has been discussed before, the quantum chemical calculations seem to give a much too short

Table 4. The contributions to the reorganization energy from each bond in the Cu(NH₃)₂(SH)(SH₂)^{0/+} complexes, calculated with the harmonic approximation in Equation 2

Bond	b_{red} (pm)	k^{red} (kJ/mol/pm ²)	b_{ox} (pm)	k^{ox} (kJ/mol/pm ²)	Δb (pm)	λ_{red} (kJ/mol)	λ_{ox} (kJ/mol)
Cu-S _{Met}	231.8	0.009	283.9	0.001	-52.1	25.2	2.1
Cu-S _{Cys}	230.8	0.021	217.0	0.042	13.8	4.0	8.0
Cu-N ₁	216.5	0.010	206.9	0.021	9.6	0.9	2.0
Cu-N ₂	220.9	0.008	206.9	0.021	14.0	1.5	4.2
S _{Met} -H _{Met1}	135.8	0.107	134.9	0.118	0.9	0.1	0.1
S _{Met} -H _{Met2}	135.0	0.115	134.9	0.118	0.1	0.0	0.0
S _{Cys} -H _{Cys}	135.3	0.112	135.3	0.115	0.0	0.0	0.0
N ₁ -H ₁₁	101.9	0.184	102.1	0.183	-0.2	0.0	0.0
N ₁ -H ₁₂	101.9	0.183	102.1	0.183	-0.2	0.0	0.0
N ₁ -H ₁₃	102.1	0.184	102.2	0.183	-0.1	0.0	0.0
N ₂ -H ₂₁	101.9	0.184	102.1	0.183	-0.2	0.0	0.0
N ₂ -H ₂₂	101.9	0.182	102.1	0.183	-0.2	0.0	0.0
N ₂ -H ₂₃	102.1	0.183	102.2	0.183	-0.1	0.0	0.0
Sum						31.7	16.3

Table 5. The contribution to the reorganization energy from each angle in the $\text{Cu}(\text{NH}_3)_2(\text{SH})(\text{SH}_2)^{0/+}$ complexes, calculated with the harmonic approximation in Equation 2

Angle	θ_{red} (°)	k^{red} (kJ/mol/deg ²)	θ_{ox} (°)	k^{ox} (kJ/mol/deg ²)	$\Delta\theta$ (°)	λ_{red} (kJ/mol)	λ_{ox} (kJ/mol)
$\text{S}_{\text{Met}}\text{-Cu-S}_{\text{Cys}}$	119.6	0.034	109.9	0.015	9.7	3.2	1.4
$\text{S}_{\text{Met}}\text{-Cu-N}_1$	118.7	0.029	93.2	0.030	25.5	18.6	19.4
$\text{S}_{\text{Met}}\text{-Cu-N}_2$	104.7	0.021	93.2	0.030	11.5	2.8	4.1
$\text{Cu-S}_{\text{Met}}\text{-H}_{\text{Met}1}$	118.0	0.028	105.6	0.024	12.3	4.2	3.6
$\text{Cu-S}_{\text{Met}}\text{-H}_{\text{Met}2}$	104.5	0.034	105.6	0.024	-1.1	0.0	0.0
$\text{S}_{\text{Cys}}\text{-Cu-N}_1$	101.2	0.025	124.3	0.024	-23.1	13.1	13.0
$\text{S}_{\text{Cys}}\text{-Cu-N}_2$	99.6	0.024	124.3	0.024	-24.7	14.7	14.8
$\text{Cu-S}_{\text{Cys}}\text{-H}_{\text{Cys}}$	102.7	0.023	99.2	0.028	3.6	0.3	0.4
$\text{N}_1\text{-Cu-N}_2$	111.6	0.034	103.1	0.041	8.4	2.4	2.9
$\text{Cu-N}_1\text{-H}_{11}$	118.5	0.042	115.4	0.050	3.1	0.4	0.5
$\text{Cu-N}_1\text{-H}_{12}$	119.6	0.032	109.8	0.058	9.8	3.1	5.6
$\text{Cu-N}_1\text{-H}_{13}$	94.9	0.033	112.1	0.049	-17.1	9.7	14.3
$\text{Cu-N}_2\text{-H}_{21}$	117.0	0.032	115.4	0.050	1.6	0.1	0.1
$\text{Cu-N}_2\text{-H}_{22}$	122.6	0.034	109.8	0.058	12.8	5.5	9.6
$\text{Cu-N}_2\text{-H}_{23}$	93.1	0.029	112.1	0.049	-18.9	10.5	17.5
$\text{H}_{\text{Met}1}\text{-S}_{\text{Met}}\text{-H}_{\text{Met}2}$	92.5	0.063	93.8	0.067	-1.3	0.1	0.1
$\text{H}_{11}\text{-N}_1\text{-H}_{12}$	107.1	0.058	106.6	0.062	0.5	0.0	0.0
$\text{H}_{11}\text{-N}_1\text{-H}_{22}$	107.4	0.058	106.6	0.062	0.8	0.0	0.0
$\text{H}_{12}\text{-N}_1\text{-H}_{22}$	107.4	0.058	105.8	0.062	1.5	0.1	0.1
$\text{H}_{21}\text{-N}_2\text{-H}_{22}$	107.0	0.058	106.6	0.062	0.5	0.0	0.0
$\text{H}_{21}\text{-N}_2\text{-H}_{23}$	107.1	0.058	106.6	0.062	0.5	0.0	0.0
$\text{H}_{22}\text{-N}_2\text{-H}_{23}$	107.6	0.058	105.8	0.062	1.7	0.2	0.2
Sum						88.9	107.6

Cu-S_{Met} bond (Ryde et al., 1996). Still, it costs only about 4 kJ/mol to elongate this bond 53 pm from the quantum chemical optimum to the experimental value and the potential curve of this bond shows two minima (one three-coordinate and one four-coordinate), explaining the failure of the harmonic model. Thus, a value less than 4 kJ/mol seems more appropriate for this contribution to the reorganization energy.

With this modification, the contribution from the bond lengths amounts to about half of the total inner-sphere reorganization energy. Only the copper ligand bonds give appreciable energies; the contribution from the other bonds is less than 0.2 kJ/mol. This may be a bit unexpected since the force constants of the former bonds are 5–20 times smaller than the other force constants. However, this difference is more than compensated by the larger change in the former bonds lengths. Among the copper ligand bonds, the Cu-S_{Cys} bond gives the largest contribution, approximately as large as the sum of the contributions of the three other bonds.

The sum of the angular contributions is much larger than the total reorganization energy. Thus, these energies are strongly overestimated. This is probably due to the harmonic approximation, which breaks down when the changes in the angles are appreciable. It is notable that all the angular force constants are of the same magnitude (0.028–0.037 kJ/mol/deg²). Therefore, the angles that change most between the two structures also give the largest contributions to the reorganization energy. Both the $X\text{-Cu-Y}$ and the Cu-X-H angles give appreciable contributions, whereas the reorganization energy from the H-X-H angles is minor. The largest angular contributions are all caused by the stronger $\text{N-H}\cdots\text{S}_{\text{Cys}}$ hydrogen bonds in the reduced structure (294–300 pm compared to 383 pm in the oxidized structure). The hydrogen bonds are more

pronounced in the reduced complex because the electrostatic attraction between the copper ion and the ligands is much weaker there. These hydrogen bonds strongly decrease the H-N-Cu(I) and $\text{N-Cu(I)-S}_{\text{Cys}}$ angles.

The dihedral angles give similar overestimation problems as the angles. However, since the dihedrals involve an ambiguity about the periodicity of the angle and since most of the dihedrals in the small model system investigated are irrelevant for the copper site in the proteins, these contributions are not included in the tables. We believe that in the protein, where most of the low-energy torsions are fixed by the folding of the protein, the contributions from the dihedral angles to the reorganization energy are minor.

In conclusion, the harmonic approximation gives very poor estimates of the inner-sphere reorganization energy. If only the bond lengths are considered, the reorganization energy is underestimated. This is true also for the other systems studied. The only system for which the bond length contributions provide a reasonable estimate of the reorganization energy is $\text{Cu}(\text{H}_2\text{O})_6^{+2}$ with a constrained geometry for the reduced complex (63 compared to 76 kJ/mol). For all other complexes, the bond length contribution is only a minor part of the total inner-sphere reorganization energy (5–40%). Of course, the reason for this is that Cu(I) prefers a tetrahedral, whereas Cu(II) assumes a more tetragonal geometry (except for some of the blue-copper models, where the geometry is trigonal). This means that there is an appreciable change in the angles of the complex, and the angles provide a major contribution to the reorganization energy. Of course, the reason why the bond contributions dominate in the constrained $\text{Cu}(\text{H}_2\text{O})_6^{+2}$ complex is that for this complex both oxidation states have the same octahedral geometry; therefore the changes in the angles are small.

Yet, if the contributions to the reorganization energy from the angles are considered by the same harmonic model, the reorganization energy is normally overestimated. The reason for this is that the changes in the angles are so large that the harmonic approximation breaks down. Thus, we must conclude that it is very hard to estimate the reorganization energy by classical methods. Clearly, direct quantum chemical calculations are much more reliable, and often also simpler to perform.

Protein strain

It is notable that our calculated reorganization energies for models of the blue-copper proteins optimized in vacuum are approximately the same as those measured experimentally on the proteins. This provides another strong argument against the entatic state and induced-rack hypotheses (Malmström, 1994; Williams, 1995). These hypotheses suggest that the protein distorts the copper coordination sphere to make the geometries of the two oxidation states of copper as similar as possible, i.e., to minimize the reorganization energy. This is not observed, however. Instead, the fully optimized geometries of the two structures in vacuum are already rather similar, and the potential surface for the internal coordinates that show the largest changes is flat, leading to a small reorganization energy. Thus, no strain is needed for the low reorganization energy.

Recently it has been suggested that the entatic nature of the blue-copper site only involves the protein restricting the approach of the axial methionine sulfur atom to the copper center both in the reduced and oxidized states (Holm et al., 1996). From our results it is hard to see any functional advantage for such constraints. We have tested cases where the Cu-S_{Met} bond is either relaxed or constrained to the (longer) length observed in crystal structures (Table 3). However, such constraints only lead to a slightly higher reorganization energy (about 4 kJ/mol).

Gray and Malmström have showed that the reduction potential of unfolded azurin is higher than that of the native protein (Leckner et al., 1997; Winkler et al., 1997; Wittung-Stafshede et al., 1998). This has been interpreted as a decrease in the coordination number of Cu(I) in the denatured protein (two- or three-coordinate). They suggest that the protein folding enforces the same coordination number (five) for the two oxidation states and that this reduces the reorganization energy of the copper site by about 170 kJ/mol. Our calculated geometries in Table 1 confirm that the Cu(I) complexes often have a lower coordination number than the oxidized complexes. Typically, the most stable structure of the reduced complexes is three-coordinate. However, for the best model of the typical blue-copper site, both the oxidized and the reduced structures are four-coordinate. Thus, the intact reduced copper site would be expected to be four-coordinate, at least in a vacuum. Yet, in a denatured protein, the copper site is exposed to an unlimited source of water molecules, which may replace the normal ligands. However, such replacements are clearly more likely for the Cu(II) ion, since this ion is harder than Cu(I) and therefore has higher affinity for the hard water ligand. Thus, it seems most likely that one or several water ligands will bind to the Cu(II) ion, thereby changing its coordination geometry from trigonal bipyramidal to distorted octahedral. The effect of denaturation on the Cu(I) geometry is harder to predict, and we confine ourselves to note that Cu(I) is more likely to retain the rather soft protein ligands than are the Cu(II) ion.

Moreover, our results do not support the suggestion that a change in the coordination number is responsible for the large increase in

reorganization energy for unfolded azurin. For several of the complexes in Table 2, we have calculated the reorganization energy both when the coordination number of the complex decreases on reduction and when the coordination number does not change. However, Cu(H₂O)₆⁺²⁺ is the only complex for which the reorganization energy increase appreciably (by 224 kJ/mol) when the coordination number changes. For all the other complexes, a change in the coordination number results in only a modest increase in reorganization energy 4–61 kJ/mol. This is probably due to the very shallow potential surface of the ligand that dissociates in the reduced complex. Therefore, we must conclude that the higher reorganization energy is unfolded azurin as well as in small inorganic copper complexes are mainly caused by the solvent reorganization energy rather than by the inner-sphere reorganization energy, and by the presence of hard water ligands in the copper coordination sphere. Thus, the role of the protein matrix of the blue-copper proteins is not primarily to restrict the geometry of the copper site, but rather to provide the appropriate ligands (nitrogen and sulfur atoms) and to protect the site from unwanted ligands (Ryde et al., 1996). Probably the protein also modulates the outer-sphere (solvent) reorganization energy by burying the copper center.

Methods and details of calculations

Eleven complexes were studied: Cu(H₂O)₆⁺²⁺, Cu(H₂O)₄⁺²⁺, Cu(NH₃)₄⁺²⁺, Cu(NH₃)₃(SH₂)⁺²⁺, Cu(NH₃)₃(SH)^{0/+}, Cu(NH₃)₂(SH)(SH₂)^{0/+}, and Cu(Im)₂(SH)(S(CH₃)₂)^{0/+} in a tetragonal coordination geometry, and Cu(NH₃)₂(SH)(SH₂)^{0/+}, Cu(Im)₂(SCH₃)(S(CH₃)₂)^{0/+}, Cu(NH₃)₂SH(NH₂HCO)^{0/+}, and Cu(Im)₂(SCH₃)(CH₃CONH₂)^{0/+} in the trigonal coordination geometry (of the oxidized form). Geometries as well as energies were calculated with the hybrid density functional method B3LYP as defined in the quantum chemical software Gaussian-94 (Frisch et al., 1995). The full geometry of the models was optimized until the maximum and root-mean-squared gradients were below 22 and 15 J/mol/pm, and the maximum and RMS changes in geometry were below 0.095 and 0.063 pm, respectively, using the standard fine integration grid. Several starting structures were tested to reduce the risk of being trapped in local minima and all chemically feasible conformations of the complexes were considered. Only the structures with the lowest energy are reported.

For copper, we used the double- ζ basis set of Schäfer et al. (1992) (62111111/33111/311), enhanced with diffuse *p*, *d*, and *f* functions with exponents 0.174, 0.132, and 0.39 (called DZpdf). For the other atoms, the 6-31G* basis sets were employed (Hehre et al., 1986). Only the pure five *d* and seven *f* functions were used. Experience shows that geometries obtained with the B3LYP approach do not change much when the basis set is increased beyond this level (Ryde et al., 1996). All calculations were run on IBM RS/6000 workstations.

For each complex studied, two different inner-sphere reorganization energies were calculated: λ_{ox} , the reorganization energy during oxidation, and λ_{red} , the reorganization energy during reduction (Fig. 1). Obviously, the total inner-sphere reorganization energy for a self-exchange reaction is the sum of these two energies, and we denote this reorganization energy by λ_i . λ_{ox} was calculated as the difference between the energy of the oxidized complex (i.e., the complex with a formal +2 charge on the copper ion) at the optimal geometry of the oxidized complex and of the reduced complex. Likewise, λ_{red} is the energy of the reduced complex at the geometry that is optimal for the oxidized complex

minus the energy of the reduced complex calculated at its optimal geometry. As can be seen from Figure 1, the two reorganization energies need not to be equal.

The B3LYP method has been shown to give accurate energies and geometries at a relatively low computational cost for transition metal complexes (Ricca & Bauschlicher, 1994, 1995; Holthausen et al., 1995; Barone et al., 1996). However, we have also compared the reorganization energies calculated with the B3LYP method with those calculated with the more accurate CASPT2 method (second order perturbation theory on a multiconfigurational complete active space wavefunction) (Andersson et al., 1990; Roos et al., 1996). For the small CuSH^- complex (using the same procedure as for our other calculations on blue-copper models (Olsson et al., 1998), this led to a difference in the reorganization energies of less than 3 kJ/mol. Similarly, calculations of the reorganization energy for $\text{Cu}(\text{NH}_3)_2(\text{SH})(\text{SH}_2)^{0/+}$ gave differences less than 4 kJ/mol, although the geometry was not optimized with the CASPT2 method (except the Cu-S_{Cys} bond length; no analytical gradients are available for the CASPT2 method).

Frequencies were calculated for all optimized geometries to ensure that the structures represent true equilibrium states. Force constants for the various bonds, angles, and torsions were calculated from the Hessian matrix using the method suggested by Seminario (1996). This method has the advantage of being fully invariant to the choice of internal coordinates. The force constants were used to calculate approximate contributions to the reorganization energy from the various internal distortions:

$$\lambda_i = \sum_b k_b^i (b_{ox} - b_{red})^2 + \sum_\theta k_\theta^i (\theta_{ox} - \theta_{red})^2 + \sum_\phi \frac{k_\phi^i}{2} (1 - \cos(n(\phi_{ox} - \phi_{red}))). \quad (2)$$

The three terms represent the energies of bond stretching, angle bending, and dihedral torsions, respectively, where b_{ox} , b_{red} , θ_{ox} , θ_{red} , ϕ_{ox} , and ϕ_{red} are the bond lengths, angles, and dihedral angles in the optimal oxidized or reduced geometry, respectively, and k_b^i , k_θ^i , and k_ϕ^i are the corresponding force constants, applying for the state i , where i is either the oxidized or the reduced state.

For several of the Cu(I) complexes, the optimal vacuum structures are two- or three-coordinate. In some cases, this is probably an artifact since the calculations have been performed in vacuum, where no intermolecular interactions are possible. This means that the structures strongly depend on internal hydrogen bonds, which often are of similar strength as the Cu-ligand interactions for the reduced complexes. In the protein or in solution, many alternative hydrogen bond partners are available that may saturate the hydrogen bond potential of the ligands even when they are bound to the copper ion. To estimate the contribution to the reorganization energy from this change in coordination number, we also optimized structures, for which the coordination number of the reduced complex was the same as for the oxidized complex. This was accomplished either by enforcing symmetry ($\text{Cu}(\text{H}_2\text{O})_6^+$) or by keeping one or a few copper-ligand bond lengths fixed. In Table 1, where all the copper-ligand bond lengths of considered complexes are collected, the actual constraints are indicated, as well as the number of imaginary frequencies in the optimized structures.

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