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Nonempirical Calculations on $(Cu^+)_2$–O_2: A Possible Model for Oxyhemocyanin and Oxytyrosinase Active Sites

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Nonempirical calculations on two copper cations (Cu(I)) interacting with a dioxygen molecule, taken as a model for the active site of oxyhemocyanin and oxytyrosinase, have been carried out to determine the most stable geometrical arrangement of this complex and its electronic structure. The results of closed-shell SCF computations show that the most stable electronic configuration of the complex is not the same for all distances between the oxygen molecule and the copper cations. Nevertheless, for the parallel as well as for the perpendicular arrangements of the two interacting entities, the lowest energy state of the complex is a singlet in agreement with EPR data. This result clearly shows that the magnetic characteristics of the active sites in question are due to a superexchange mechanism taking place through the sole dioxygen molecule. Moreover, the most stable conformation of the complexes obtained from this study for different Cu–Cu distances, corresponds to a location of the oxygen molecule between the two metal ions in agreement with the available experimental data on $[(L_nCu)_2–O_2]^{2+}$ model systems. Our results show also that this trans arrangement induces an important lengthening of the O–O bond. This feature, which is in agreement with Raman and X-ray data, is not found when the complex has the cis-μ-dioxo conformation and provides further evidence for the validity of our simplified model. The electronic structure obtained for the most stable geometrical arrangement is discussed in relation to the experimenal spectroscopic data concerning model complexes as well as oxyhemocyanin and oxytyrosinase active sites themselves and with respect to possible mechanisms of action for the second of these enzymes.

Introduction

The binuclear active site of hemocyanin and tyrosinase, two metalloproteins (respectively found in arthropods and molluscs and widely spread among plants and mammals) that both bind one oxygen molecule per active site, has been thoroughly studied by different spectroscopic methods such as Raman,[1] UV,[2,3] EPR,[1b,c,3] and EXAFS.[4] But the detailed geometry and electronic structure of their active oxy form remain to be solved[5] since no crystal X-ray diffraction nor theoretical studies have been reported for these two enzymatic systems; therefore, the models[3b,c,4b,c,6] proposed in the literature have been deduced from the sole available spectroscopic data. Thus it is the large decrease of the O–O Raman frequency occurring upon the binding of dioxygen to hemocyanin that indicates an increase of its bond length due to electron transfer from the cations to this ligand,[7] the EXAFS data[4] giving a confirmation of the existence of a vacancy in the copper ion d shell of oxyhemocyanin and oxytyrosinase. This result tends to show that, upon oxygen binding, an electron transfer takes place from the two Cu(I) cations of the enzyme's native form to dioxygen. Therefore, a peroxoanion is formed, and the degree of oxidation of the coppers increases. But the absence of any EPR signal due to oxyhemocyanin or oxytyrosinase[1b,c,3a] suggests that the two Cu(II) ions are coupled through a superexchange mechanism,[8-10] which implies the presence of a bridging ligand between the two metal ions, while the electron density around the copper ions of *Panulirus interruptus* hemocyanin[11] does not exhibit evidence for a bridging ligand. Solomon and collagues[2] proposed the cis-μ-dioxo (Figure 1B) arrangement as more probable than the monooxo one (Figure 1A) from the comparison of the UV spectra of the oxy form of the enzymes with the values of the interaction energy of the two $O_2^{2-} \rightarrow$ Cu(II) transition dipole moments and of the ratio of their sum and difference for the two cases considered.

On the other hand, the two $[(L_nCu)_2–O_2]^{2+}$ complexes for which geometry has been completely determined exhibit a $(Cu^I)_2$–O_2 geometrical pattern "rather" different from the retained model since they correspond to a trans-μ-dioxo[12] or μ-η^2:η^2 conformation[5] (Figure 1C). These complexes are as EPR silent as oxyhemocyanin and oxytyrosinase in spite of the absence between the two metal atoms of any briding ligand aside from O_2, and their UV spectra exhibit an absorption band in the 500–600-nm range, which can be assigned to an $O_2^{2-} \rightarrow$ Cu(II) charge-transfer absorption. Moreover, in Kitajima's complex,[5b] which exhibits a $Cu^+\cdots Cu^+$ distance very close to those measured in hemocyanin,[11] all the spectroscopic data available are very similar to those of the enzymatic oxy form.

With all this in mind we decided to carry out quantum-mechanical calculations on a model for the binuclear copper active site of oxytyrosinase and oxyhemocyanin in order to have at our disposal a set of reliable results which could (i) give information on the relative stabilities of the different possible geometrical arrangements as well as on their respective electronic structures and therefore contribute to the understanding of the factors responsible for the antiferromagnetic behavior of these systems and (ii) be used later as reference for a more complete study of tyrosinase mechanisms of action in which one of us has become recently interested.[13]

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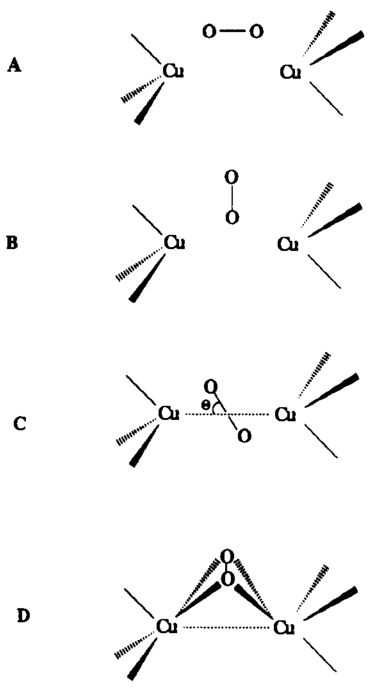

Figure 1. Geometrical arrangements and parameters of the $(Cu^+)_2$–O_2 complexes studied: (A) μ-monoxo; (B) cis-μ-dioxo; (C) trans-μ-dioxo or μ-η^2:η^2; (D) η^2:η^2.

These computations, which will use the recent X-ray results on *P. interruptus* hemocyanin[11] and the indications given by the EXAFS data[4] on the geometrical parameters concerned, will attempt to account for the different spectroscopic features characteristic of these systems. In order to have calculated values free from possible artifacts due to the approximations and/or the parametrization linked to all semiempirical methods, they will be carried out at the ab initio level. Furthermore, such results will be useful benchmarks for the semiempirical procedures that will have to be used in the study of a more complete model of the active sites including the different ligands and substrates or inhibitors. Because of the number of electrons involved in such binuclear systems, we had to limit the nonempirical calculations to two naked copper cations interacting with a dioxygen molecule. In the present case, the neglect of the metal ligands should hopefully not imply major qualitative differences for the results obtained: (i) the X-ray and EXAFS data have shown that in the considered enzymes the environment of both copper ions is almost identical; (ii) since the metal atoms have a d^{10} configuration in the deoxy form (the starting point for the formation of the complex), the splitting of d orbitals by the ligand field is quite small[14] (vide infra). In addition, such nonempirical results will provide, through the extent of the agreement between experiment and theory, an estimation of the nature and the importance of the ligand contribution to the problem studied.

Computational Input and Procedures

For oxygen, we used the 3-21G* basis set[15] to which we added Dunning's[16] diffuse p functions. These diffuse functions, which are necessary in calculations concerning anions,[17] were introduced since, according to the active-site model proposed in the literature, the dioxygen is in the peroxo form. For the copper cations, we used the 3-21G basis proposed by Dobbs and Hehre[18] in which the exponents of the 3d, 4sp′ and 4sp″ have been modified in order to take into account that the two copper cations have a d^{10} configuration in hemocyanin and tyrosinase[3b] while the published data concern a $3d^94s^2$ excited state of the copper atom. The exponent values used for the 3d and 4sp atomic orbitals, which are those having the largest relative difference when going from the ^2S to the ^2D state,[19] have been calculated from those proposed by Dobbs and Hehre using the $^2S/^2D$ ratio in Tatewaki and Huzinaga's work[19] with the exception of the 4sp″ exponent. In this case the value retained is 0.031 38.[20] With these modifications, the energy of the Cu(I) cation is improved by 0.74 eV with respect to the value obtained with Dobbs and Hehre's original exponents. Moreover, we obtain smaller electron populations in the 4sp atomic orbitals. For the atomic orbitals of the water molecules of the $(Cu^+(H_2O)_3)_2$ system that we have considered, we used the same minimal basis set as in ref 21.

The calculations have been carried out by using two different programs, a modified version of MONSTERGAUSS[22] and HONDO (7.0).[23] The former was used for the determination of the geometrical parameters in the complex and the atomic orbital populations. The latter, which is less appropriate for the calculation of the copper orbital populations since it works with the six Cartesian d orbitals and therefore introduces a spurious 3s orbital,[24] was used for the computation of the configuration interaction contribution to the energy and of the vibrational frequencies. For this last quantity, the computational procedure followed is the one developed by Komornicki and Mc Iver.[25]

The first determination of the optimum geometrical parameters of the different possible complexes has been carried out for the closed-shell single-determinant wave function (RHF), since several ab initio studies on dimeric copper(II) complexes have shown that such results give a reasonable zeroth order approximation for the computation of antiferromagnetic coupling constants.[10,26] The molecular orbitals obtained for the most stable parallel (cis-μ-dioxo) and perpendicular (which will have a μ-monoxo, a trans-μ-dioxo, or a μ-η^2:η^2 conformation) arrangements (depicted in Figure 1A–C) were used as input for configuration interaction calculations (CI). For each of the two complexes, the CI treatment was made for three electronic configurations, i.e. the closed-shell ground state plus the singlet and the triplet obtained by exciting one electron from the highest occupied to the lowest empty orbital of the single-determinant closed-shell wave function. For each of these three electronic configurations the CI includes all single and double excitations involving orbitals 27–42 (orbitals 36 and 37 being the closed-shell RHF HOMO and LUMO) with respect to the reference configuration. The number of orbitals thus introduced into the configuration interaction process is small compared to the size of the molecular basis (16 and 94, respectively). However, it includes dioxygen valence orbitals and the six highest copper d orbitals plus a set of empty 4s and 4p copper orbitals. This choice of orbitals and degree of excitation, which introduces 35 266 configurations, takes into account the orbitals that are at the origin of the leading contributions to the energy lowering. It also corresponds to the complete CI between the orbitals determining the spin coupling, namely the dioxygen π and the copper d orbitals involved in the (anti)-ferromagnetic coupling. In order to study the influence of the neglected orbitals, we have, for the most stable trans- and cis-μ-dioxo conformations of the complexes, carried out open-shell restricted Hartree–Fock (ROHF) calculations of the singlet and triplet states followed by a CI taking into account the orbitals and excitations described above. We ran also a two-reference-determinant closed-shell SCF calculation (ROHF–GVB[22,23]), followed by the same CI, since such a wave function is the one corresponding to the singlet state of the two unpaired electrons localized on the two copper cations, provided that the two molecular orbitals concerned are the g and u combinations of copper atomic orbitals.

For the calculation of the absorption energies, the orbitals used as input are those of the ROHF–GVB computations since they were found to give the electronic state of lowest energy for both conformations of the

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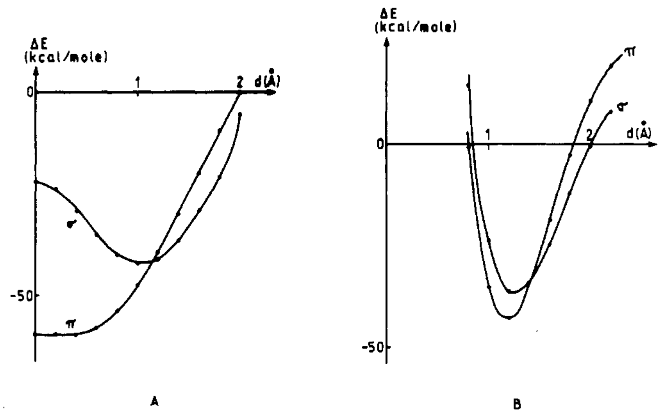

Figure 2. Binding energy (in kcal/mol) of $(Cu^+)_2$ with O_2 as a function of d, the distance between the centers of the O–O and $Cu^+–Cu^+$ lines (see Figure 1): (A) μ-monoxo approach (Figure 1A); (B) cis-μ-dioxo approach (Figure 1B).

complex. In addition the CI was limited to single and double excitations from the two-determinant reference state and limited to orbitals 31–40 in order to avoid the numerous low-lying d → d transitions which are not relevant to the problem studied.

In the series of computations dealing with the "parallel versus perpendicular" dioxygen binding, the Cu–Cu distance is taken equal to 3.7 Å, the first value measured in crystalline hemocyanin.[11a] Since the EXAFS data[4b] have shown that, in different hemocyanins, the $Cu^+–Cu^+$ distance undergoes only small variations upon oxygen binding, we can reasonably expect that keeping this parameter constant would have only minor effect on the results obtained.

With the basis set used throughout this work, the O–O bond length giving the best energy at the SCF level for the molecular $^3\Sigma_g$ ground state is 1.18 Å, a value close to the experimental one (1.21 Å[27]). The O–O distance retained for the exploratory approaches was 1.20 Å in order to have a value close to experiment but which also takes into account the tendencies of the basis set.

Results and Discussion

In order to estimate the ligand field stabilization energy of the copper cation d orbitals we carried out calculations on $(Cu^+)_2$ on one hand and $(Cu^+(H_2O)_3)_2$ on the other. The calculated splitting of the d orbitals increases from 0.29 to 0.98 eV upon the addition of the ligands. This very limited variation produced by the ligand field, which, in addition, does not modify the relative energy ordering of the d orbitals, is mainly due to the splitting of their u/g combinations. Therefore, the ligand field should not be a determining factor for the electronic structure of the complex formed upon oxygen binding.

When O_2 is interacting with the copper cations its two π_g orbitals are no longer degenerate. Using the modification of MONSTERGAUSS introduced for a study of Zn^{2+} interacting with various ligands[21] or the AC option of the program, which allows one to modify the orbital occupation pattern, it is possible to have as initial guess a wave function in which one of the dioxygen π_g orbitals is doubly occupied and the other empty. For the perpendicular and parallel approaches depicted in Figure 1A,B, Figure 2 reports the value of the $(Cu^+)_2-O_2$ interaction energy as a function of d, the distance between the middle of $Cu^+–Cu^+$ and O–O bonds, the four atoms being coplanar. For both geometrical arrangements, the curve labeled σ corresponds to an electronic structure in which the dioxygen π_g orbital located in the plane of the complex, and therefore belonging to the σ-orbital system, is doubly occupied while the one that is a combination of the oxygen p orbitals perpendicular to the plane of the complex and hence of π symmetry is empty. The curves labeled π correspond to the reverse situation. We see from Figure 2 that for large values of d the most stable closed-shell electronic structure is the one with the two unpaired electrons of free dioxygen in the $\pi_g(\sigma)$ orbital. For smaller values of d, this electronic structure is less stable than the one in which O_2 carries four π electrons. For both parallel and perpendicular approaches the crossing of the σ and π energy curves occurs for similar distances and interaction energy values; in addition, the overall shape of the two σ curves are identical. On the contrary, the variation of the energy of the complexes with four π electrons on O_2 depends strongly on the topology of the approach, the perpendicular one being the most stable. Finally, the most energetically favorable arrangement of $(Cu^+)_2-O_2$ occurs when the oxygen molecule is located between the two copper cations, since for both type of complexes any distortion from the planar arrangement of the four atoms produces an energy increase.

The optimization of the O–O bond length carried out for the most stable arrangements of the complexes show that the increase of the oxygen–oxygen distance from 1.20 to 1.28 Å improves the energy of the perpendicular complex while destabilizing the parallel one. A further energy gain is obtained for the former conformation when the angle between the $Cu^+–Cu^+$ and O–O directions is changed from 90 to 80°. In the case of the parallel conformer (Figure 1B), the binding energy is not improved by the presence of an OH^- taken as model of the "endogenous" bridging ligand.[3] For the d value corresponding to the best energy of the complex on Figure 2B (d = 1.2 Å), O_2 binding energy to $(Cu^+)_2-OH^-$, with Cu–O(H) = 2 Å, is found to be positive (27.7 kcal/mol). Therefore, it appears that the most stable theoretical structure, which corresponds to Figure 1C, is not in agreement with the model proposed for the oxy form of the enzymes[6] but is fully consistent with X-ray data[5,12] on model complexes.

Since the results of geometry optimizations are strongly dependent upon the degree of refinement of the computational procedure used,[28] we reoptimized the O–O bond length and the θ angle (Figure 1C) by using the ROHF–GVB procedure, in order to tentatively improve the calculated geometrical parameters of the complex. At this point of the study, we could reasonably expect that the order of stability for the two types of arrangements would not be modified by changes in the computational procedures; therefore the optimization was carried out only for the most stable trans conformation. A similar treatment was not attempted for the open-shell singlet and/or triplet, nor was CI included, because of the additional computer time which would thus have been required. It is for this last geometry (l_{O-O} = 1.48 Å, θ = 80°) that the energy of the three different electronic configurations of the trans-μ-dioxo complex has been calculated. It is worthwhile to emphasize that the calculated value of 80° for θ is intermediate between those measured in the model complexes (89 and 55°), a feature that appears very satisfactory since our results concern a $Cu^+–Cu^+$ distance of 3.7 Å, which is between the corresponding quantity in the compounds experimentally studied (3.6 and 4.3 Å, respectively). Therefore, it appears that the conformation obtained from theory is intermediate between the trans-μ-dioxo and the μ-η^2:η^2 arrangements described, which for the sake of simplicity we call the trans conformation. In the case of the cis-μ-dioxo arrangement, the computations are reported for d = 1.2 Å and l_{O-O} = 1.2 Å.

The comparison of the sets of energy values (reported in Table I) concerning the two geometrical arrangements of the complex is, for each of the electronic structures studied, in favor of the trans conformer, and this difference is found at all the computational levels that we have considered. The tabulated values concerning the three electronic configurations built from the closed-shell single-determinant SCF wave function (RHF) show that their energy orderings are not identical in both conformations. The open-shell singlet is the least stable in both conformers while the relative energies of their closed-shell singlet and triplet are different. This situation is modified by the configuration interaction since the corresponding results give for both arrangements lower energy values for the closed-shell singlet (i.e. the electronic state in which the leading configurations are closed-shell determinants) than the triplet state, in agreement with the absence of any EPR signal.[3] This result shows that the dioxygen molecule

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Table I. Energy (in au) of the Lowest Singlet (with a Closed- or an Open-Shell Configuration) and Triplet States Calculated at the SCF and CI Levels[a] for the Cis- (Figure 1A) and Trans-Dioxo (Figure 1C) Geometrical Arrangements of the $(Cu^+)_2$–O_2 Complex

	cis conformation			trans conformation		
	singlet			singlet		
	closed shell	open shell	triplet	closed shell	open shell	triplet
RHF	−3409.7941	−3409.6105	−3409.7532	−3409.8082	−3409.5347	−3409.8919
RHF + CI	−3409.8335	−3409.7689	−3409.7827	−3409.9351	−3409.8771	−3409.9203
ROHF–(GVB)	−3409.8905	−3409.7758	−3409.8902	−3410.0209	−3410.0181	−3410.0188
ROHF–(GVB) + CI	−3409.9036	−3409.8672	−3409.9024	−3410.0229	−3410.0224	−3410.0207

[a] The description of the different computational steps and the abbreviations used are given in the text.

can be the bridging ligand responsible for the superexchange mechanism between the two copper ions not only when the complex has the trans conformation[5,12] but also if the arrangement is cis-μ-dioxo.[6]

However, the examination of the molecular orbitals and the CI eignevectors shows that the most stable electronic structures of the two conformers are significantly different. When the arrangement is cis, the closed-shell single-determinant lowest empty orbital, which is singly occupied in the open-shell singlet and triplet, is mainly a combination of the copper 4s and dioxygen $\pi_g(\sigma)$ orbitals. In the case of the trans conformer, the copper contribution comes principally from the 3d orbitals while the influence of the oxygen molecule is reduced.

Because of these differences, the configuration interaction produces only a moderate mixing for the cis conformation, while for the trans arrangement it leads to a singlet that exhibits similar large contributions from two closed-shell configurations, namely the one given by the SCF procedure and the one in which the HOMO is empty and the LUMO filled. Since these two orbitals correspond to the symmetrical and antisymmetrical combinations of the copper d_{xy} orbitals, the electronic structure described for this singlet is in agreement with the one expected for two antiferromagnetically coupled unpaired electrons.[9]

At this point we must mention that when the RHF molecular orbitals are used, open-shell singlets with an unpaired electron in a dioxygen π orbital and the other in a combination of copper atomic orbitals are found in the energy range between the values reported in Table I for the closed- and open-shell structures. For this cis conformer, a $\pi \rightarrow \sigma^*$ triplet is also found to be located between the closed-shell singlet and the triplet having the symmetry of the problem studied. Their values are not reported since (i) they are not relevant to the anti/ferromagnetic coupling of the two Cu(II) and (ii) after CI, their energies do not correspond to the lowest open-shell states anymore.

The values of Table I corresponding to the ROHF results show that the open-shell singlets are, as expected,[29] less stable than the corresponding triplets but that this computational step produces a general energy gain of more than 3 eV with respect to the RHF value concerning the same electronic structure. The magnitude of the energy lowering shows the importance of the rearrangement of the occupied orbitals when the single-determinant closed-shell LUMO is occupied; it is this result that induced us to run the ROHF–GVB computations. The results thus obtained show that for both geometrical arrangements of the complex this type of singlet state is the most stable electronic structure. The two orbitals which are alternatively occupied correspond to the g and u combinations of copper d orbitals. This result confirms that the complex exists as a singlet with two unpaired electrons coupled through a superexchange mechanism. The configuration interaction does not modify the relative stabilities of the different electronic structures of the cis conformer, but in the case of the trans arrangement, it produces for the open-shell singlet an energy gain large enough to render it more stable than the triplet state by about 450 cm^{-1} and less stable than the ROHF–GVB–CI singlet by only 93 cm^{-1}.

The examination of the ROHF results concerning the open-shell singlet of the trans conformer shows that this computational procedure does lead to a wave function in which each of the two singly occupied orbitals are localized on one copper ion. For this electronic structure a configuration interaction calculation limited to the two singly occupied orbitals leads to an energy of −3410.020 au, which is lower than the ROHF value obtained for the corresponding triplet. This result is in agreement with the expected contribution of the Cu^{2+}–Cu^0 ionic forms to the superexchange mechanism,[8,9] a feature that does not appear explicitly from the ROHF–GVB and ROHF–GVB–CI computations on the closed-shell electronic structure. In order to examine the stability of the CI energy value reported in Table I with respect to the choice of the molecular orbitals used as input, we followed a procedure used by Mougenot et al.[30] for the study of divanadium complexes. In the case of the trans conformer, when the triplet ROHF orbital is used, the lowest singlet-state energy is −3410.0225 au while the triplet is calculated at −3410.0208 or −3410.0203 are when the ROHF–GVB and open-shell ROHF singlet MO are respectively given as input. These results are undoubtedly in favor of the singlet ground state of the complex. The different values of Table I, which are in favor of the trans conformation of the complex, clearly show that, for both arrangements, the ground state is a singlet because an antiferromagnetic coupling between the two copper ions takes place through the sole dioxygen molecule. This result is in agreement with the large ground-state exchange interaction obtained by Ross and Solomon[31] from SCF–Xα–SW calculations for the side-on μ-η^2:η^2 peroxide-bridged copper dimer studied by these authors. There is then no requirement for any additional ligand, which according to our results tends to inhibit dioxygen binding (vide supra); this constitutes an a posteriori argument in favor of our simple model. This result could have been expected from the values of the Cu–O–Cu angles, which are 110 and 130°, respectively, and within the range for which the coupling is antiferromagnetic.[8,24,32] Furthermore, it is worth stressing that in the more stable trans conformation each copper ion has two oxygen atoms at 1.87 and 2.11 Å, a result fully consistent with the EXAFS data for oxytyrosinase.[4c]

Table II. Oxygen Atomic Population in the Ground State of the Cis- (Figure 1A) and Trans-μ-Dioxo (Figure 1C) Conformations of the $(Cu^+)_2$–O_2 Complex

	cis-μ-dioxo	trans-μ-dioxo
HONDO		
RHF	8.08	8.24
RHF + CI	8.23	8.54
ROHF–GVB	8.42	8.60
ROHF–GVB + CI	8.34	8.59
MONSTER		
RHF	8.12 ($d^{9.66}$)	8.33 ($d^{9.47}$)
ROHF–GVB	8.65 ($d^{9.04}$)	8.72 ($d^{9.01}$)

However, the analysis of the energy calculation results and of the optimized geometrical arrangement of the complexes does not provide any information on the process responsible for the Cu(I) → Cu(II) transition, which is experimentally observed upon the formation of the $(Cu^+)_2$–O_2 complex. It could take place either through a $Cu^+ \rightarrow O_2$ charge transfer or through a 3d → 4s promotion. This last mechanism could be responsible for the hole

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Table III. Calculated Values of the Vibrational Frequencies (cm^{-1}), and Intensities for the Trans-μ-Dioxo Conformation of the $(Cu^+)_2$–O_2 Complex

ν	22	85	240	313	406	752
intensity	10	2	1	3	41	116
IR/Raman	R	IR	IR	IR	R	R

observed[4] in the copper 3d shell since we are dealing in the present case with cations. Previous studies have shown on one hand that neutral metal atoms do not transfer more than one electron to the ligands in dicopper complexes[9,26] and on the other that upon dioxygen binding on metal surfaces a single electron is transferred to O_2.[33] Moreover, a recent study has shown that a $3d^94s^1$ bonding scheme as well as a $3d^{10}$ scheme can be obtained for the $CuCH_3^+$ cation.[34]

The values reported in Table II show that at each computational level used the $(Cu^+)_2 \rightarrow O_2$ charge transfer is more important in the trans-μ-dioxo arrangement. This difference is only to a limited extent due to the longer O–O bond length in this conformer since, at the RHF level for O–O = 1.2 Å, the total oxygen population is 8.115 and 8.234 in the cis and trans arrangements respectively. The respective average Cu–O distances in the two conformers (2.23 Å in the cis and 1.99 Å in the trans) may be at the origin of this charge-transfer differential efficiency.

The tabulated values show also that the oxygen populations obtained from the RHF–CI and ROHF–GVB wave functions are larger than those of the corresponding RHF runs. But the configuration interaction treatment on the ROHF–GVB wave functions produces a very limited variation of the atomic charges. Thus the population analysis given by ROHF–GVB computations carried out with the MONSTERGAUSS program (that is without the 3s orbitals) should give a reasonable representation of the electron distribution in the system. We see that in the more stable trans conformation ≃1.4 electrons have been transferred from the metal cations to dioxygen but that only 9 electrons are located in the copper 3d shell, a result in excellent agreement with EXAFS data.[4] Therefore ≃0.3 electrons are shifted from the 3d to the 4sp shell upon the formation of the complex. This shift is somewhat larger in the case of the cis conformer, 0.33 versus 0.28 in the trans complex. It is also noteworthy that for both conformers the calculated $Cu^+ \rightarrow O_2$ charge transfer between the RHF and ROHF–GVB results concerns principally the 3d shell (Table II).

The vibrational frequencies and intensities values calculated at the RHF level for the trans-μ-dioxo conformation are reported in Table III. The computations could definitely not be carried out at the ROHF–GVB level because of the computer time requirement linked to the size of the basis set; for such runs the symmetry of the complex could not be used because of program limitations. However, the values of Table III show clearly that the results reproduce correctly the low-frequency shift for the O–O vibration upon complex formation, since we obtain a value of 752 cm^{-1} while the calculated value for the O_2 ground state is 1732 cm^{-1}, the corresponding experimental values being 742[1a] and 1550 cm^{-1}.[25] At this point, we must underline the fact that the value of the frequency shift is determined not only by the variation of the O–O bond length but also, to some extent, by the modification of the electronic structure of O_2 upon complexation since for an isolated oxygen molecule having a bond length of 1.48 Å (as in the complex), the calculated value of the O-O stretching frequency is 507 cm^{-1}. We see also from Table III that the Raman bands of the complex aside from the O–O stretch are located at 406 (Cu–O) and 22 (Cu–Cu) cm^{-1} and thus could not be observed experimentally (cf. Figures 1 and 2 in ref 1a and ref 1c, respectively). We are aware that, in the oxy form of the experimentally studied hemocyanins,[3] the symmetry of the $(Cu^+)_2$–O_2 complex is partly altered; however, the IR bands at 240 and 313 cm^{-1} (the second of which corresponds to the Cu–O stretch) can have only a very weak Raman intensity because of the $\simeq C_{2h}$ geometry of the system. Therefore, the absence of an observable Cu–O stretching mode in the experimental Raman spectrum is an additional argument in favor of trans-μ-dioxo arrangement of the atoms in the complex.

Table IV. ROHF–GVB Frontier Orbital Symmetry Species and Energies (in hartrees) for the Cis (Figure 1A) and Trans-μ-Dioxo (Figure 1C) Conformations of the $(Cu^+)_2$–O_2 Complex

orbital[a]	cis-μ-dioxo		trans-μ-dioxo	
31 ($O_2\pi_u^\sigma$)	A_1	−1.058 97	B_u	−0.968 43
32 ($O_2\pi_u^\pi$)	B_1	−0.968 82	A_u	−0.916 54
33 ($O_2\sigma$)	A_1	−0.952 20	A_g	−0.874 36
34 ($O_2\pi_g^\sigma$)	B_2	−0.719 81	A_g	−0.835 37
35 ($O_2\pi_g^\pi$)	A_2	−0.668 38	B_g	−0.745 78
36 (Cu 3d)	A_1	−0.616 02	B_u	−0.654 52
37 (Cu 3d)	B_2	−0.534 28	A_g	−0.531 81
38 (Cu 4s)	A_1	−0.396 73	A_g	−0.381 97
39 (Cu 4sp)	B_2	−0.371 59	B_u	−0.362 14
40 (Cu 4sp)	A_1	−0.279 91	B_u	−0.237 17
41 (Cu 4pπ)	B_1	−0.263 19	A_u	−0.236 39
42 (Cu 4pπ)	A_2	−0.228 80	B_g	−0.234 80

[a] Main location of the orbital is given in parentheses.

Table V. Theoretical Characteristics of the First Magnetically (m) and Electrically (e) Allowed Transitions in the Cis (Figure 1A) and Trans (Figure 1C) Conformations of the $(Cu^+)_2$–O_2 Complex[c]

cis						trans					
sym[a]	orbtl[b]				ΔE, eV	sym[a]	orbtl[b]				ΔE, eV
B_1	35	36	37	38	1.27 (m)	B_g	35	38			4.03 (m)
A_2	35	38			1.34 (m)	B_u	34	36	37	38	4.46 (e)
A_1	34	36	37	38	1.55 (e)	B_g	35	36	37	39	5.03 (m)
A_2	35	36	37	39	2.00 (m)	A_g	34	38			5.11 (m)
B_1	35	39			2.02 (m)	A_g	34	36	37	39	5.38 (m)
B_2	34	38			2.06 (e)	B_u	34	39			6.26 (e)

[a] Symmetry of the excited state. [b] Open-shell orbitals of the excited states main configuration(s). [c] For oxyhemocyanin[2] the corresponding measured values are 2.18 and 3.59 eV for the electrically allowed bands and 2.58 eV for the magnetically allowed one.

Table VI. Binding Energies (ΔE) and Geometrical Parameters in $(Cu^+)_2$–O_2 Complexes with Different Cu–Cu Distances (R)

R, Å	ΔE, kcal/mol	l_{O-O}, Å	θ, deg	z,[a] Å	l_{Cu-O}, Å
3.1	−184.5	1.52	90	0.5	1.80
3.4	−190.5	1.50	90	0.0	1.85
3.7	−174.3	1.48	80	0.0	1.87
4.0	−159.3	1.48	60	0.0	1.75
4.3	−147.5	1.48	50	0.0	1.77

[a] z = 0.0 indicates coplanarity of the four atoms.

Large extended polarized basis sets[35] have to be used to calculate with some accuracy quantities concerning excited states of bimetallic complexes. Therefore, even if, as currently supposed by experimentalists, the imidazole ligands do not contribute significantly to the absorption spectra of oxyhemocyanin and oxytyrosinase,[2,3] we cannot expect more than qualitative indications from excited-state energies calculated with the 3-21G basis used throughout this work, even if polarization and diffuse Gaussian functions are present on the oxygens. Inspection of their symmetry polarization and energy of the frontier molecular orbitals, from the 31st to the 42th reported in Table IV, shows that in both conformers the dioxygen $\pi_g(\pi)$ orbital is the highest one, below the copper d orbitals in which are the coupled unpaired electrons, followed by the $\pi_g(\sigma)$, the σ, and the two π_u orbitals, the other copper d orbitals being at lower energy. This location of the σ and π_u dioxygen orbitals is different from the one obtained, on larger complexes, by Ross and Solomon[31] from SCF-Xα–SW calculations. But these authors' results are in agreement with those obtained from our closed-shell single-determinant computations.

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Table VII. Experimental Values of the Geometrical Parameters and CD and Absorption Bands in Peroxo Dinuclear Copper Complexes and Oxyhemocyanin

				spectral band, eV			
R, Å	l_{O-O}, Å	θ, deg	l_{Cu-O}, Å	UV/vis	CD	UV/vis	ref
3.22	*a*	*a*	1.99	*a*	*a*	*a*	31
3.31	*a*	*a*	1.97	*a*	*a*	*a*	31
3.56	1.41	89	1.90	2.25	2.58	3.55	5
3.6–3.7	*a*	*a*	*a*	2.18	2.58	3.59	2
4.36	1.43	55	1.85	2.10	*a*	2.36	12

[a] Not reported.

Table VIII. Calculated Values (in eV) of the CD (m) and Absorption (e) Bands in the $(Cu^+)_2$–O_2 Complexes Characterized in Table VI

R = 3.1 Å	R = 3.4 Å	R = 3.7 Å		R = 4.0 Å	R = 4.3 Å
		90°	80°		
5.41 (e+m)	5.24 (m)	3.87 (m)	4.03 (m)	3.99 (m)	3.75 (m)
5.79 (m)	5.44 (e)	4.31 (e)	4.46 (e)	4.38 (e)	3.86 (m)
6.34 (e+m)	6.29 (m)	4.93 (m)	5.03 (m)	4.41 (m)	4.10 (e)
6.42 (m)	6.46 (m)	5.00 (m)	5.11 (m)	4.79 (m)	4.24 (m)
6.89 (e+m)	6.80 (m)	5.30 (m)	5.38 (m)	5.09 (m)	4.72 (m)
7.22 (m)	7.89 (e)	6.18 (e)	6.26 (e)	5.59 (e)	4.82 (e)

This similarity illustrates the limited influence of the nitrogen ligands on the spacing and ordering of the molecular orbitals primarily influenced by the complex formation. The data in Table IV show also that orbitals 36–40 are of σ symmetry; therefore, the lowest excited state should correspond to an electrically forbidden $\pi \rightarrow \sigma^*$ transition, a feature which is not in agreement with the available experimental data. The results reported in Table V show that the values obtained from the CI treatment retained for these quantities do not quantitatively fit the experimental data[2,5,12] (Table VII) since (i) for both conformers an electrically forbidden transition is the lowest one, (ii) for the cis arrangement the calculated transition energy values are much smaller than the measured ones while they are too large in the case of the trans conformation, and (iii) several magnetically allowed bands are calculated between the electrically allowed transitions. However, the analysis of the leading contributions to the calculated transitions shows that the long wavelength absorption band has a theoretical intensity weaker than that of the second peak and that both transitions correspond to O → Cu charge transfers, features satisfactory in relation to the measured spectra.[2,3,5,12] The intensity difference between the two bands is due to the totally different orbital occupation patterns in the two corresponding excited states. For the lower energy state, the configuration with the largest coefficient corresponds to a double excitation with respect to both ground-state determinants; therefore, the associated transition dipole is small. On the contrary, the main contributions to the higher energy state come from configurations corresponding to a single excitation with respect to one of the two ground-state components and are consequently associated with a larger transition dipole moment.

The poor agreement of the calculated absorption spectra occurring for both geometrical arrangements of the complex tends to show that the contribution of the imidazoles to the UV spectra cannot be neglected. We are there reaching the limits of validity for our model ignoring the protein ligands.

In order to get additional support for the relevance of our model, we have optimized the geometrical arrangement of the complex with Cu–Cu distance values covering the range reported for enzyme models in the literature, that is 3.1–4.4 Å.[5b,12,36] These computations are carried out at the ROHF–GVB level since the results reported in Table I show that the combination of the two closed-shell determinants give results that appear qualitatively stable with respect to further computational refinements. The values reported in Table VI indicate that the largest binding energy of the complex is obtained when the Cu–Cu distance (R) is equal to 3.4 Å. For this value of R, the complex is planar and the O–O bond is perpendicular to the Cu–Cu direction (θ = 90°, Figure 1C). We see also that upon decrease of the intercopper distance, the oxygen molecule tends to move out of the original plane. The optimal arrangement is reached for 0.5-Å displacement when R = 3.1 Å (Figure 1D), the O–O and Cu–Cu directions remaining perpendicular. This result is in agreement with the structure proposed by experimentalists for oxygenated complexes featuring a short Cu–Cu distance[37] (R = 3.2–3.4 Å). For increasing values of R, our results indicate that the rotation of the oxygen molecule increases as well (decreasing θ value). This finding, which is in complete agreement with experimental data (Table VII) demonstrates that θ is primarily determined by the distance between the copper cations since the experimental data concern complexes with a varying number of different ligands. However, the comparison of calculated and measured values of θ shows that our calculations tend to slightly overestimate the rotation of O_2. It is worth noticing that this rotation is responsible for the very limited variations of the Cu–O distance (Table VI and VII) in the corresponding cases considered. In addition, these computations indicate that the oxygen electronic population remains approximately constant since it varies by less than 0.025 electron for the whole set of optimized complexes studied.

The calculated values of l_{O-O} reported in Table VI show that, if theory gives correctly the increase of the O–O bond length which occurs upon dioxygen complexation, the measured stretches (0.20–0.22 Å) are smaller than the calculated ones (0.30–0.34 Å). However, the limited numerical difference (~0.1 Å) between experimental and theoretical values may reasonably be attributed to the neglect, in our computations, of the metal ligands, which most certainly led to some overestimation of the perturbation due to the copper ions. The experimentally studied complexes exhibit a net charge smaller than one because of the ligand nitrogen lone pair → metal cation charge transfer.

If we now consider the calculated values of the absorption bands obtained for the different Cu–Cu distances (Table VIII), we see that the calculated splitting between the circular dichroism bands is, except when R = 4.3 Å, 2–5 times smaller than the energy gap between the two O → Cu electrically allowed transitions, a feature which could explain why this phenomenon has not, to our knowledge, been reported by experimentalists. More important in relation with experimental data is the fact that the calculated transition energies are shifted to longer wavelengths when the Cu–Cu distance increases, this variation being more pronounced for the high-energy (short-wavelength) peak. This result is in good agreement with experiment since the corresponding observed bands are shifted from ≃2.2 and 3.6 eV[5,2] to 2.1 and 2.4 eV[12] when the intercopper distance increases from 3.6–3.7 to 4.4 Å (Table VII).

The two sets of numbers reported for R = 3.7 Å show that the decrease of θ produces a short wavelength shift of the magnetically and electrically allowed bands. However the tabulated values show clearly that despite the θ decrease that occurs upon increasing R, an overall bathochromic effect is obtained. Since the calculated bathochromic shift is in agreement with the available data on complexes in which the number and/or structure of the nitrogen ligands are different, it implies that the Cu–Cu distance is the determining factor not only for the orientation of the dioxygen molecule between the two copper cations but also for the absorption spectrum pattern. Regarding the excited states, it can be seen from the list of their most important configurations that the $O_2^{2-} \rightarrow$ Cu(II) back-donation takes place mainly into the metal 4s orbital and only to a more limited extent into the 3d shell.

Conclusion

The present theoretical study indicates that the ground state of oxyhemocyanin and oxytyrosinase can be a singlet for which the antiferromagnetic coupling between the two Cu(II) ions takes place through the sole peroxo anion and does not require any supplementary endogenous ligand, in agreement with the observations of Jacobson et al.[12a] and Kitajima et al.[5b] on model com-

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plexes. However, it appears that our calculations do give for the singlet–triplet separation a value that is smaller than the experimental one since, from the values of Table I, we obtain 257 and 550 cm^{-1} for the cis and trans conformers, respectively, while the measured quantity is >600 cm^{-1}.[6c] The corresponding values obtained by Ross and Solomon[31] from their Xα computations are quite larger (1850 and 5660 cm^{-1}). At this point, it is worth stressing that, in the case of the cis-μ-dioxo conformation, previous authors have introduced an OH^- to model a possible bridging ligand. Since both Solomon's and our own results lead to an antiferromagnetic coupling more than two times larger for the μ-η^2:η^2 conformation than for the cis-μ-dioxo arrangement, it is clear that the extra bridging ligand does not contribute significantly to the Cu(II)–Cu(II) antiferromagnetic coupling. On the other hand, the most stable geometrical arrangement we find for Cu_2–O_2 is similar to those observed for the above-cited model complexes which are, as are both enzymes, EPR silent and bind dioxygen. In particular, the calculated value of the "short" Cu–O distances (1.75–1.87 Å) is very close to those measured in the model complexes (1.85–1.99 Å). The proximity of these values is in favor of the importance of the Cu–Cu distance for the determination of the geometrical arrangement of dioxygen between the two metal cations. The agreement between the theoretical and experimental structures (vide supra) suggests that while the nitrogen ligands are essential for the screening of the $Cu^+\cdots Cu^+$ repulsion, their influence on the $(Cu^+)_2$–O_2 complexation is not determinant per se; this statement gets good support from the location of dioxygen in very different systems such as cobalt diporphyrins.[38] Actually, the exact structure and number of the ligands determine the $Cu^+\cdots Cu^+$ distance through the cross attraction between each copper and the second metal ion ligands.[39]

Considering the different spectroscopic data concerning oxyhemocyanin, oxytyrosinase, and their model complexes, it is worth emphasizing that the comparison between the experimental and theoretical absorption spectra tends to show that, in binuclear copper enzyme active sites and complexes, the wavelength of the O → Cu charge-transfer band is primarily determined by the value of the metal–metal distance. Morever, the calculation gives a very good account for the experimental EXAFS and Raman data as well as the absence of an EPR signal. The analysis of the atomic populations in the complex exhibits a highly dipolar situation with strongly negative oxygen atoms, potentially very attractive toward labile protons, while the importance of the positive charge on the copper ions makes them good candidates for the fixation of oxygen ligands such as phenols and catechols. In addition, this important charge on the metal (>1.5) suggests that Cu(II) could, as Zn^{2+} does,[21,40] considerably decrease the deprotonation energy of such ligands and therefore lead to a transfer of the proton from the hydroxyl group to the peroxo anion. A complex exhibiting features very similar to a highly reactive hydroperoxo intermediate proposed by experimentalists[41] is thus obtained.[42]

In conclusion, the geometrical pattern and electronic structure of the complex, obtained from the present study, fulfill the main requirements expected for tyrosinase models: two Cu(II) cations antiferromagnetically coupled through a peroxo–dioxygen anion. Furthermore, it is worth noticing that a dioxygen displacement by a few tenth of an ångstrom hardly destabilizes the complex since $\Delta d = \pm 0.4$ Å produces an energy variation of only 0.2 kcal/mol (Figure 2A). Such a flexibility can contribute to the well-known low substrate specificity of tyrosinase.[43]

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Registry No. O_2, 7782-44-7; Cu_2^+, 37381-77-4; oxytyrosinase, 9002-10-2.

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