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Charge transfers influence on the spin ground state of manganese and iron superoxide dismutases: a DFT study on a model of the reduced active site interacting with O_2^-†

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From DFT and time-dependent DFT calculations on Mn^{II}SOD and Fe^{II}SOD active site models interacting with O_2^- we have determined that metal-to-ligand charge transfers stabilise the S = 2 and S = 5/2 spin states as ground spin states for the [Mn^{II}SOD-O_2^-] and [Fe^{II}SOD-O_2^-] model complexes, respectively. These charge transfers are ruled by the electronic configuration of the metal ion, and they can be determinant in the catalysis reaction.

Superoxide dismutases (SOD) are a group of metalloenzymes that catalyse the disproportionation of the superoxide anion radical (O_2^-) by successive reduction and oxidation of the metal at the active site.[1] Iron (FeSOD) and manganese (MnSOD) superoxide dismutases present very similar active sites, with an identical set of coordinating ligands.[2]

We recall here that the magnetic exchange coupling constant (J) is composed of a ferromagnetic and an antiferromagnetic term. Usually, the antiferromagnetic term is the predominant one. In Anderson's model, both metal-to-ligand or ligand-to-metal charge transfers (MLCT and LMCT, respectively) contribute to the antiferromagnetic term of the J constant.[3] Sometimes, these transfers can contribute in a different way to the J value, as predicted in the double exchange or in the Goodenough's model, where the ferromagnetic interactions are favoured.[4] In this sense, the relevance of the contribution of CT to the J constant has been analysed by Solomon in models of Mn(III)-O-Mn(III) complexes and deoxyhemocyanine.[5]

We have performed DFT calculations for active site models of both reduced and oxidized FeSOD and MnSOD proteins as well as of the reduced proteins interacting with O_2^- (see Fig. 1).‡ In this work we demonstrate that the nature of the magnetic exchange coupling between the paramagnetic centers (O_2^- anion and the divalent metal ion) is ruled by MLCT processes.

In these M(II)-O_2^- systems, a large overlapping between the single occupied molecular orbital (SOMO) of the O_2^- anion and the proper t_{2g} and e_g SOMOs of the metal ion should lead to a strong antiferromagnetic contribution. In the model of the manganese active form, our DFT calculations show that the S = 2 ground state is strongly stabilized (J = −218.3 cm^{-1}).§ However, for the corresponding iron model our results evidence a strong ferromagnetic exchange coupling (J = +164.3 cm^{-1}) which stabilizes the S = 5/2 state as the ground state. In order to explain such spin exchange interactions, the CT processes have to be investigated since the enzymatic function involves one electron transfer from the metal ion to the substrate and *vice versa*.

In the case of the manganese model, the electronic configuration of the Mn(II) ion and the O_2^- anion, together with the orbitals that can be involved in a charge transfer from the metal ion to the substrate (MLCT) are shown in Fig. 2. In the

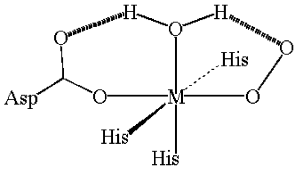

Fig. 1 Geometrical topology of the studied model corresponding to an active form of the enzyme. Asp = aspartate, His = histidine and M = Fe or Mn divalent ions.

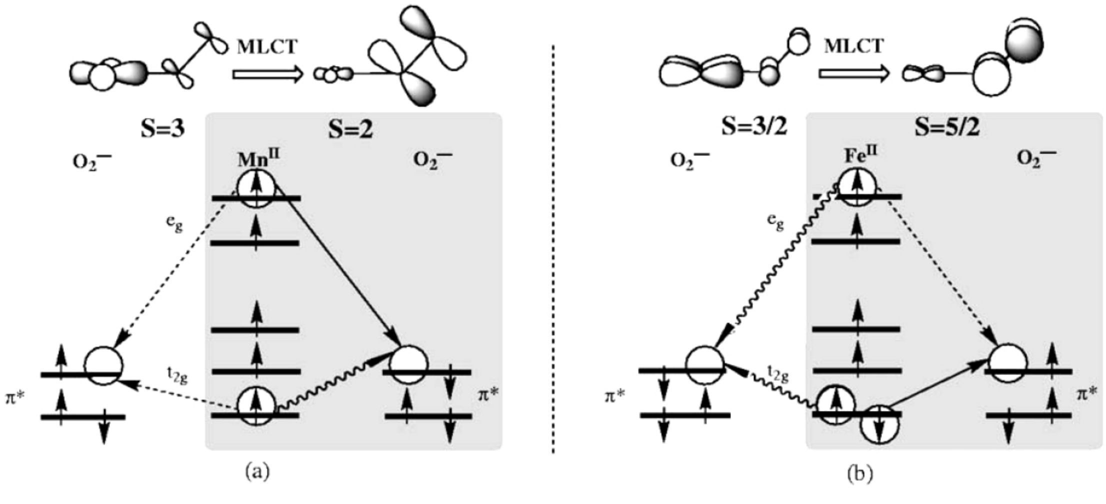

Fig. 2 Metal to ligand charge transfer (MLCT) processes depending on the spin state and on the orbitals involved in the processes for: a) manganese and b) iron models. The circles and arrows show, respectively, the involved orbitals and the electron transfer processes. Solid, wavy and dashed arrows indicate most probable, less probable and forbidden transfers. The spin state where the CT is more favourable is shown within a grey square.

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† Electronic supplementary information (ESI) available: construction of the models; configuration interactions. See http://www.rsc.org/suppdata/cc/b2/b211427e/

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ferromagnetic $S = 3$ state, the CT is forbidden since the O_2^- anion cannot accept any other α electron on its SOMO (Fig. 2). However, in the $S = 2$ state, an electron transfer from the Mn(II) ion to the π* O_2^- orbital is possible. This MLCT decreases the spin density on the O_2^- anion and, since an antibonding molecular orbital is filled, the O–O distance of the substrate increases. We confirm that the spin density of the O_2^- ligand is lower and its O–O bound distance is higher in the $S = 2$ than in the $S = 3$ state. Results herein clearly show that the electron transfer takes place as we have proposed (see Table 1). Moreover, from comparison of the spin density value on the metal ion in the $S = 2$ state with the corresponding value in the reduced and oxidized forms of the MnSOD model (see Table 1),¶ we conclude that this is just a partial electron transfer. Also, the value of the O–O distance in the $S = 2$ state is intermediate between those in the optimised O_2^- and O_2^{2-} anions. This partial electron transfer stabilizes the $S = 2$ state, and thus increases the antiferromagnetic exchange coupling predicted by the superexchange model. Furthermore, in the valence bond framework, an extra stabilization of the $S = 2$ ground state would come from the interaction between this M^{II}-O_2^- state and a M^{III}-O_2^{2-} (peroxo form) $S = 2$ excited state energetically close.† Time-dependent density functional (TD-DFT) calculations on the $S = 2$ state evidence that there is an excited state at 16 000 cm^{-1}, which is close enough to allow a weak interaction with the $S = 2$ ground state.§ In fact, the energy gap between the pure M^{III}-O_2^{2-} and M^{II}-O_2^- states is smaller, since DFT calculations provide an energy equivalent to that of the states already mixed.

The electronic configuration of the Fe^{II}-O_2^- system and the orbitals involved in a possible MLCT process are shown in Fig. 2. In the $S = 5/2$ and $S = 3/2$ states, it is allowed to transfer a β and an α electron, respectively, from the metal $t_{2g}(d_{xy})$ orbital to the π* orbital of the substrate. Nevertheless, this charge transfer in the $S = 3/2$ state leads to an excited configuration for the metal ion, whereas in the $S = 5/2$ state, the electron transfer leads to a stable electronic configuration for both the iron ion and the substrate. In the $S = 3/2$ spin state, other MLCT from α d orbitals are also allowed which lead us to a very high-excited electronic configuration for the metal ion and/or for the substrate. Moreover, the contribution of these excited states in the stabilization of the ground $S = 3/2$ state is negligible since the overlapping between the involved iron d orbital and the empty α π* orbital of the substrate is near to zero. As in the manganese model, we have performed TD-DFT calculations in order to obtain the energy of the excited $S = 5/2$ and $S = 3/2$ states, corresponding to a Fe^{III}-O_2^{2-} species, which could eventually interact with the ground $S = 5/2$ and $S = 3/2$ states respectively and stabilize them. The Fe^{III}-O_2^{2-} excited $S = 5/2$ state is only at 13 200 cm^{-1} (753 nm), while the excited $S = 3/2$ states are situated at much higher energy (28 400 cm^{-1} and > 42 000 cm^{-1}). Therefore, the strong stabilization of the $S = 5/2$ state counterbalances the antiferromagnetic contribution predicted by the superexchange model, and a global interaction of ferromagnetic nature is obtained.† As in the case of the manganese model, these conclusions are backed up by the calculated values of the atomic spin densities and the O–O bond lengths (see Table 1), which point out that a greater charge transfer occurs in the $S = 5/2$ spin state.

Table 1 Electronic, energetic and geometrical results obtained for the M^{II}-O_2^- active site model, with M^{II} = Mn or Fe.¶

Model	S^a	$\rho_M{}^b/e^-$	$\rho(O_2^-)^c$	$d_{oo}{}^d$/Å	J^e/cm^{-1}
Mn^{II}-O_2^-	3	4.77	1.05	1.392	-218.3^e, -234.8^e, -164.9^e
	2	4.57	−0.76	1.414	
Fe^{II}-O_2^-	5/2	3.86	0.88	1.423	$+164.3^e$, $+190.4^e$, $+121.9^e$
	3/2	3.73	−0.92	1.385	

[a] Spin moment for the spin state; [b] metal atomic spin density; [c] spin density on the substrate; [d] O–O interactomic bond length in the substrate; [e] exchange coupling constants calculated from the minimum of the potential curve, zero-point energy and free energy at room temperature, respectively.

In summary, the different electronic configurations of the Mn(II) and Fe(II) ions induce different MLCT which support antiferro- and ferromagnetic coupling respectively for [Mn^{II}SOD-O_2^-] and [Fe^{II}SOD-O_2^-] model complexes. In this way, the product of the reductive reaction (that is, the oxidized forms of the enzymes) and the [M^{II}SOD-O_2^-] complex present the same multiplicity in the ground spin state. Therefore, the reactive spin state is the ground state, and no extra energy is needed to populate it. We propose here that this electronic factor is determinant in the MnSOD and FeSOD enzymatic processes.

Notes and references

‡ Among the possible coordination modes of the metal center to the substrate, we have studied the conformation shown in Fig. 1, that leads to an active species.[6] The built models have been considered as the starting point for a geometrical optimisation by DFT calculations. These optimisations have been carried out for all species in all studied spin states. See ESI.†

§ All theoretical calculations were carried out with the hybrid B3LYP method,[7] as implemented in the GAUSSIAN98 program.[8] Double-ζ and triple-ζ quality basis sets proposed by Ahlrichs and co-workers have been employed for non-metal and metal atoms, respectively.[9] Also, two extra polarization p functions have been added for the metal atoms. The broken symmetry approach has been used to evaluate the antiferromagnetic state energy as reported previously.[10] The zero-point energy and the free energy corrected to room temperature have also been calculated. The atomic spin densities and charges were obtained by a Natural Bond Orbital (NBO) analysis.[11]

¶ These results must be compared with the ones we have obtained for the optimised models of the reduced ($\rho_{Mn} = 4.78$ and $\rho_{Fe} = 3.74$) and oxidized ($\rho_{Mn} = 3.80$ and $\rho_{Fe} = 4.19$) active sites of the enzyme, and for superoxide ($\rho(O_2^-) = 1.00$ and $d_{OO} = 1.399$ Å) and peroxide ($\rho(O_2^{2-}) = 0.00$ and $d_{OO} = 1.520$ Å) anions.

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