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Rapid Report

Change in electron and spin density upon electron transfer to haem

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Abstract

Haems are the cofactors of cytochromes and important catalysts of biological electron transfer. They are composed of a planar porphyrin structure with iron coordinated at the centre. It is known from spectroscopy that ferric low-spin haem has one unpaired electron at the iron, and that this spin is paired as the haem receives an electron upon reduction (I. Bertini, C. Luchinat, NMR of Paramagnetic Molecules in Biological Systems, Benjamin/Cummins Publ. Co., Menlo Park, CA, 1986, pp. 165–170; H.M. Goff, in: A.B.P. Lever, H.B. Gray (Eds.), Iron Porphyrins, Part I, Addison-Wesley Publ. Co., Reading, MA, 1983, pp. 237–281; G. Palmer, in: A.B.P. Lever, H.B. Gray (Eds.), Iron Porphyrins, Part II, Addison-Wesley Publ. Co., Reading, MA, 1983, pp. 43–88). Here we show by quantum chemical calculations on a haem *a* model that upon reduction the spin pairing at the iron is accompanied by effective delocalisation of electrons from the iron towards the periphery of the porphyrin ring, including its substituents. The change of charge of the iron atom is only approx. 0.1 electrons, despite the unit difference in formal oxidation state. Extensive charge delocalisation on reduction is important in order for the haem to be accommodated in the low dielectric of a protein, and may have impact on the distance dependence of the rates of electron transfer. The lost individuality of the electron added to the haem on reduction is another example of the importance of quantum mechanical effects in biological systems.

Keywords: Density functional; Haem charge; Quantum chemistry

The location of the surplus electrical charge upon reduction of a haem group has been unclear. On the one hand, it has been suggested that the charge may be distributed over the whole haem structure [4], but in some electrostatic calculations the charge has been confined to the central iron atom [5]. The latter convention may appear reasonable at first sight because it is known that the single unpaired spin in a low-spin ferric haem is largely localised to the d_{xz}, d_{yz} (d) orbitals of the iron, and that it becomes paired on reduction of the haem to the ferrous state [1–3]. For this spin to become paired, the iron needs to acquire one extra electron of opposite spin. However, our preliminary semi-empirical quantum chemical calculations on a haem *a* model suggested that the unit charge by which the oxidised and reduced states of the haem differ is evenly distributed throughout the porphyrin ring. To unravel this question, we performed the more rigorous density functional calculations to be reported here.

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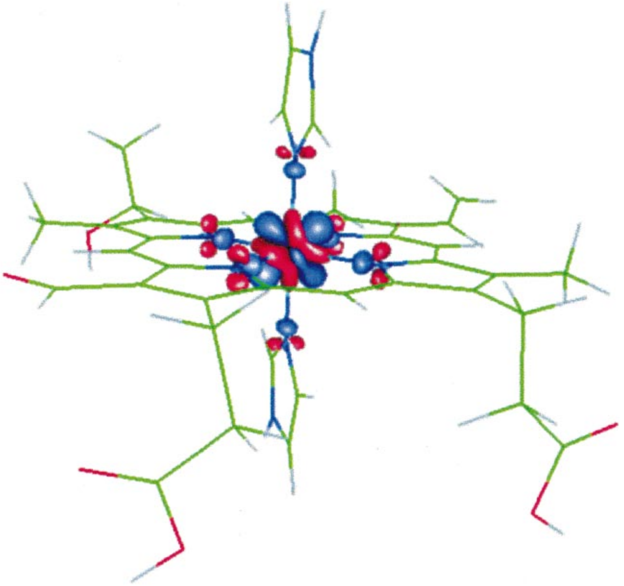

Fig. 1. Electron density difference between the oxidised and the reduced forms of haem *a*. Blue colour represents areas with more electrons in the reduced form as compared to the oxidised form, while red areas represent more electrons in the oxidised form. The density plots were made using the gOpenMol package [9].

Haem *a* is the low-spin, bis-imidazole-ligated cofactor of many cytochrome *c* oxidases [6], which catalyse most of the biological consumption of O_2. Like many other haems, it is essential for electron transfer, which involves continuous shuttling between the oxidised (ferric) and reduced (ferrous) states. Here we use a haem *a* model where the propionate substituents are protonated and the long hydroxyethylfarnesyl side chain is truncated into a hydroxyethyl group.

The molecular structures of the low-spin ferrous ($S = 0$) and low-spin ferric ($S = 1/2$) haem *a* were fully optimised using the B3LYP hybrid density functional [7,8], and their atomic structures were found to be very similar (not shown). The difference between the charge distributions of the ferric and ferrous form is obtained by straightforward subtraction. Fig. 1 shows that reduction of the ferric haem occasions significant reorganisation of the charge density around the central iron. As the only visible changes of the charge density appear to be in the vicinity of the iron atom, one might again expect the net unit change of charge upon reduction to be located in this area, too. However, as seen in Fig. 2, integration of the charge density difference reveals that the net

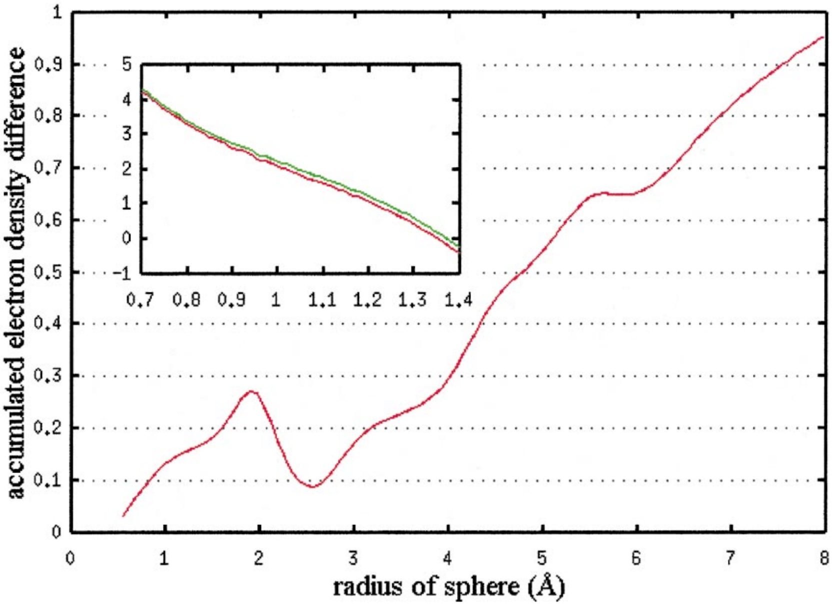

Fig. 2. The accumulated electron density difference between the reduced and oxidised form of haem *a*. The central iron atom defines the origo. (Inset) The iron charge in the reduced (red) and the oxidised (green) haem *a* as a function of the atomic radius.

change of charge at the iron is only about 10–15% of an electron (see below). Thus, in Fig. 1, the excess charges on iron in the ferrous and ferric forms nearly cancel; the ferrous form has a slight excess of negative charge along the Fe-N bonds, as compared to ferric, while the latter has more electrons on the remote side of the nitrogens. This polarisation of the charge around the ligating nitrogens results in the local maximum at about 1.9 Å in the accumulated electron density difference plot (Fig. 2). At a distance of 2.5 Å from the central iron atom, the accumulated electron charge is again only 10% of an electron larger for the reduced than for the oxidised haem. For larger distances from the central iron atom, the accumulated electron density difference function increases approximately linearly with the radius, and the whole added electron is found only when the integration sphere includes the entire haem structure. The extra charge of the reduced haem is thus completely dispersed over the entire molecule, including even the propionate substituents. The change of charge of the haem iron is far less than the unit change in oxidation number, as noted earlier for transition metal complexes [10,11]. The same qualitative picture is obtained from Mulliken population analysis. It is clear that reduction of the haem must not be considered to be a reduction of the iron ion, but a reduction of the entire haem molecule.

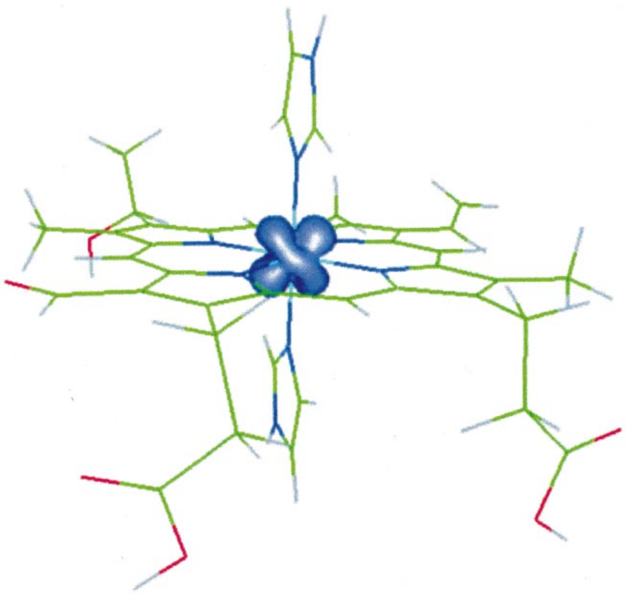

Fig. 3. Spin density for the low-spin ($S = 1/2$) ferric form of haem *a*.

The spin density of the ferric form of haem groups is known to be located almost entirely at the central iron [1–3]. This suggests that even though oxidation of the haem removes an electron that is evenly dispersed over the whole structure, it should nevertheless result in a very localised concentration of the spin. Integration of the spin density of the ferric haem, i.e. the difference between the spin-α (spin up) and spin-β (spin down) density, indeed shows that the entire unpaired electron is found in a d_{xz}, d_{yz} orbital inside the covalent radius of the iron (Fig. 3). Thus, while the charge density difference is scattered over the entire haem molecule, the spin density is completely localised at the iron.

Our preliminary studies show that these results can also be extended to include five-coordinated high-spin haems. The properties studied here are also insensitive to the chosen density functional and basis set. Calculations at the BP density functional theory level [12–14] qualitatively reproduce the results of the more involved B3LYP method [7,8], the only difference being that the charge polarisation at the ligating nitrogens is less pronounced.

The present result, though perhaps surprising, can nevertheless be rather easily understood because spin and charge densities are two independent observables at the many-body level of theory. As the exchange interaction between electrons with parallel spins is attractive [15], it is energetically more favourable to locate the unpaired electron of the ferric haem at the iron with its many electrons than to delocalise it over the entire molecule. In the ferric haem, one 3d electron has been removed resulting in diminished 3d-3d repulsion. The haem iron can then attract electrons from the surrounding porphyrin and thereby almost completely compensate for the lost electron. As spin-α and spin-β electrons are attracted equally, the spin at the iron is not affected. Conversely, when the haem is reduced the open shell at the iron will be filled, and due to the 3d-3d repulsion the surplus electron charge will become scattered as pairwise delocalisation of electrons towards the periphery minimises Coulomb repulsion. This simple mechanism would explain the observed localisation of the spin density at the iron and the simultaneous delocalisation of the charge density over the entire haem molecule.

The mechanism is probably not unique for haems, and may well appear also in other transition metal redox systems. In addition, it may be noteworthy that Johansen [16] showed a difference in the distribution of spin and charge density in a metal-to-ligand charge transfer transition of permanganate.

The spread of the surplus charge throughout the haem structure upon acceptance of an electron assures minimisation of the electrostatic energy penalty in low dielectric surroundings, particularly within a biological membrane where haem *a* naturally resides. This effect should thus contribute to the electron affinity (redox potential) of the ferric haem [17]. Our finding may also favour the convention adopted by Dutton et al. [18], where the rates of haem–haem electron transfer reactions are related to the edge-to-edge distance between donor and acceptor, rather than to the distance between the iron atoms. This would require fast electron exchange between the relevant orbitals of the haem iron and those of the porphyrin, which seems quite plausible due to the mixing of the electron accepting d_{xz}, d_{yz} orbital of low-spin ferric haem iron with the π orbitals of the porphyrin [1–3].

Methods

The molecular structure, the electron charge and spin densities of the ferrous and the ferric oxidation states of the haem *a* model were calculated at the density functional theory (DFT) level using both a gradient-corrected local density approximation (BP) [12–14] and a hybrid functional (B3LYP) [7,8]. The calculations were performed with the TURBOMOLE program package [19] using its standard basis sets [20–22]. Most calculations used a split valence basis, with polarisation functions present on all atoms except hydrogens. The validity of this basis set was confirmed by calculations with a triple-ζ basis, with polarisation functions on all atoms, including hydrogens.

The difference between the electron densities of the two oxidation states was obtained by subtracting the charge density of the oxidised haem *a* from the reduced form. The spin density was obtained as the difference between the spin-α and spin-β contributions to the charge density of oxidised haem *a*. When comparing the electron densities, the same molecular structure was used for both oxidation states. The choice of structure, reduced or oxidised, does not affect the results.

The electron densities were evaluated in discrete equidistantly distributed Cartesian grid points. The spatial distributions of the charge and spin density differences were studied by performing explicit numerical integration of the discrete representation. The accumulated charge or spin density inside a sphere with radius r is then obtained as

$$e_{\mathrm{tot}} = \frac{1}{N}\frac{4\pi}{3}r^3\sum_{i}^{N}\rho_i \tag{1}$$

where N is the number of integration points inside the sphere, ρ_i is the calculated density in the grid point i, and e_{tot} is the accumulated electron density. To improve the accuracy of the numerical integration, the deep core orbitals of the heavy atoms (1s for C, N and O, as well as 1s, 2s and 2p for Fe) were not considered.

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