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A comparison of the inner-sphere reorganisation energies of cytochromes, iron-sulphur clusters, and blue copper proteins

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Running title: Comparison of inner-sphere reorganisation energies

Abstract

Inner-sphere reorganisation energies have been calculated for a number of models of six-coordinate iron porphyrins (with varying axial ligands), using the density functional B3LYP method. If the axial ligands are uncharged, the reorganisation energy is very low, 5-9 kJ/mole. If one of the axial ligands is charged, the reorganisation energy is higher, 20-47 kJ/mole, but such sites are normally not used in electron carriers. The former reorganisation energies are appreciably smaller than what was found four blue copper proteins (62-90 kJ/mole), the dimeric Cu_A site in cytochrome *c* oxidase and nitrous oxide reductase (43 kJ/mole), and six different types of iron-sulphur clusters with one, two, or four iron atoms (40-75 kJ/mole), even if these vacuum energies are typically halved inside the protein (as a result of hydrogen bonds and solvation effects). Therefore, the cytochromes seem to have the inherently lowest inner-sphere reorganisation energy of the three commonly used electron carriers. All three types of sites have reduced the reorganisation energy by using a delocalised charge and N- and S-donors (rather than O-donors) as metal ligands. Moreover, iron is a more appropriate metal for electron transfer than copper, because Fe(II) and Fe(III) prefer the same coordination number and geometry and give weaker bonds than copper. The low-spin state of the cytochrome has a 20 kJ/mole lower reorganisation energy than the corresponding high-spin site. Moreover, ring strain in the porphyrin reduce the changes in the Fe-N_{Por} distances by 5 pm and therefore the reorganisation energy by 8 kJ/mole.

Keywords: blue copper proteins, cytochromes, iron-sulphur cluster, quantum chemical calculations, reorganisation energy.

Introduction

In nature, there are three common types of metal sites whose function is solely to carry an electron from one place to another, viz. cytochromes, iron-sulphur clusters, and blue copper proteins [1,2]. The metal environment is quite different in these three sites. The cytochromes consist of an iron ion bound to a porphyrin ring. Two axial ligands complete the octahedral coordination sphere. During electron transfer, iron alternates between Fe(II) and Fe(III), both in the low-spin state [3]. Several types of cytochromes have been classified in biological system, depending on the substituents of the porphyrin ring, the axial ligands, and the number and arrangement of the haem groups in the protein (cytochromes *a*, *b*, *c*, *f*, etc.) [2]. The axial ligands are typically His or Met, but proteins an amino terminal, carboxylate, or Tyr are known [3]. In addition, several haem proteins are five-coordinate but their function is catalysis or transport, rather than electron transfer [3].

Iron-sulphur clusters consist of iron ions surrounded by four sulphur ions, either cysteine thiolate groups or inorganic sulphide ions. Regular clusters with one (rubredoxins), two, three, or four (all three ferredoxins) iron ions are known, as well as a number of more irregular clusters (occasionally with other protein ligands than cysteine) [4-7]. The individual iron ions are in the high-spin state, but the spins normally couple antiferromagnetically to a low total spin.

The blue copper proteins, finally, consist of a copper ion bound to a cysteine and two histidine residues in an approximate trigonal plane. In addition, there may be one or two axial ligands, typically methionine (most proteins), glutamine (the stellacyanins), or a backbone carbonyl group (in addition to methionine in the azurins) [8-10]. A variation to this theme is provided by the Cu_A site, present in cytochrome *c* oxidase and nitrous oxide reductase, which consists of two copper ions bridged by two cysteine sulphurs, and each coordinated by a histidine residue and an axial ligand, either methionine or a backbone carbonyl group [11,12].

The reduction potentials of the individual proteins within these groups vary much, but in general blue copper proteins have the highest potentials (+180-1000 mV [13]; all reduction potentials are relative the standard hydrogen electrode) and the iron-sulphur clusters have the lowest potentials (-700 to +400 mV [6]) [1]. The cytochromes have intermediate reduction potentials, ranging

between -300 and +470 mV [3,14]. This is succinctly illustrated by the electron transport chain in oxidative phosphorylation, where the first complex (at the lowest potential) contains several iron-sulphur clusters, the second complex involves one iron-sulphur cluster and several cytochromes, whereas the final complex (at the highest potential) contains two haem groups and two copper centres [1].

According to the semiclassical Marcus theory [15], the rate of electron transfer is given by

$$k_{ET} \quad \frac{2}{\hbar} \frac{H_{DA}^2}{\sqrt{4 \quad RT}} \exp \quad \frac{(\quad G^0 \quad)^2}{4 \quad RT} \quad . \qquad (1)$$

Here, H_{DA} is the electronic coupling element, which is a function of the overlap of the wavefunctions of the two states involved in the reaction. It depends on the delocalisation of the electron to be transferred in the metal site and the protein matrix between the two active sites. G_0 is the free energy of the reaction (the reduction potential difference between the donor and acceptor sites), which is a function of the electronic and solvation energies of the two sites. is the reorganisation energy, i.e. the energy associated with relaxing the geometry of the system after electron transfer. It can be divided into two parts: inner- and outer-sphere reorganisation energy, depending on which atoms are relaxed. For a metal-containing protein, the inner-sphere reorganisation energy is associated with the structural change of the first coordination sphere, whereas the outer-sphere reorganisation energy involves structural changes of the remaining protein as well as the solvent.

Several groups have studied the reduction potential energy of both cytochromes [16-24] and iron-sulphur clusters [25-30] with theoretical methods. For example, it has been shown that the reduction potential of related proteins and mutants can be predicted with reasonable accuracy (average error 50 mV) if the crystal structure is known [28]. If the structure is not know, the results are worse [20].

The outer-sphere reorganisation energy of cytochromes has also been estimated by theoretical methods [7,17,22,24,31-33]. However, no direct estimate of the inner-sphere reorganisation energy seems to be available. Instead, it is usually assumed to be negligible compared to the outer-sphere

contribution or is estimated from the experimentally measured change in metal-ligand bond distances [15,17,22,32,33]. From a theoretical point of view, the inner-sphere reorganisation energy is most interesting, since it is the only parameter in Eqn. (1) that is a function only of the metal site, i.e. it does not depend on the detailed structure of the protein. Therefore accurate calculations of realistic models of the isolated metal centres can be expected to give results that allow for a general comparison of the various types electron-transfer proteins.

Recently, we have published a detailed discussion of the inner-sphere reorganisation energy of the blue copper proteins and the Cu_A dimer [34-36], as a step in a continuing investigation of these proteins [37-42]. We have also studied the inner-sphere reorganisation energy of several types of iron-sulphur clusters, both in vacuum and in the protein [43]. In this paper, we complete this investigations by studying the inner-sphere reorganisation energy of a number of cytochrome models. This gives us the opportunity to contrast the various sites and discuss how they have optimised their electron-transfer function.

Methods

Quantum chemical geometry optimisations were performed with the density functional method B3LYP (unrestricted formalism for open-shell systems), as implemented in the Turbomole software [44,45]. Hybrid density functional methods have been shown to give as good or better geometries as correlated ab initio methods for first-row transition metal complexes [46-48], and the B3LYP method in particular seems to give the most reliable results among the widely available density functional methods [49]. In all calculations, we have used for iron the double- basis set of Schäfer et al. (62111111/33111/311) [50], enhanced with one *d*, one *f*, and two *p* functions with exponents 0.1244, 1.339, 0.134915, and 0.041843, respectively. For the other atoms, we have employed the 6-31G* basis sets [51]. Only the pure 5 *d* and 7 *f*-type functions were used. Calibrations have shown that geometries obtained with this approach do not change much when the basis set is increased [40,52]. The full geometry of all models was optimised until the change in

energy between two iterations was below 10^{-6} Hartree (2.6 J/mole) and the norm of the internal gradients was below 10^{-3} a.u. (0.053 pm or 0.057°). No symmetry restraints were imposed.

For the cytochromes, we studied iron porphine (a porphyrin ring without any substituents) with two axial ligands. Our studies of ferrochelatase have indicated that the porphyrin side chains have a very small influence on the structure of the haem group [53]. As axial ligands, we have used $S(CH_3)_2$, imidazole, CH_3NH_2, SCH_3^-, $C_5H_6O^-$, and CH_3COO^- as models of methionine (Met), histidine (His), an amino terminal, cysteine (Cys), tyrosine (Tyr), and a carboxylate group (Asp/Glu), respectively. All complexes were assumed to be in the low-spin state (a closed-shell singlet for Fe^{II} and a doublet for Fe^{III}), in accordance with experiments [54].

The inner-sphere reorganisation energy was estimated in the same way as for the blue copper proteins [35]: The reorganisation energy for the oxidised complex ($_{ox}$) was calculated as the difference in energy between the Fe(III) system at its optimal geometry and at the optimal geometry of the Fe(II) system. Likewise, the reorganisation energy for the reduced complex ($_{red}$) was calculated as the energy of the Fe(II) system at its optimal geometry minus the energy of the Fe(II) system calculated at the geometry optimal for the oxidised complex. This is actually the definition of the reorganisation energy [15], except that it should be a free energy whereas we calculate energies at 0 K. Yet, the effect of entropy and temperature is small for the inner-sphere part, as test calculations on blue-copper models have shown [55]. The Marcus equation (1) describes the relation between the total reorganisation energy and the rate constants, which are measured experimentally. This relation is based on several approximations. The total inner-sphere reorganisation energy for a self-exchange reaction ($_i$) is the sum of $_{ox}$ and $_{red}$. In variance to the outer-sphere reorganisation energy, the inner-sphere reorganisation energy is independent of the actual geometry of the complex between donor and acceptor sites and has therefore a functional significance [42].

Results and Discussion

Geometry and reorganisation energy of the cytochrome models

We have calculated geometries and inner-sphere reorganisation energies of iron porphine with seven different sets of axial ligands. The results are collected in Tables 1-2. The prototypal combination of axial ligands is His-Met, which is found in most *c*-type cytochromes (class I, IIb, and IV) and in cytochrome b_{562} [3,56]. As can be seen in Table 1, the Fe-N_{His} distance decreases by 3 pm when the site is reduced, whereas the Fe-S_{Met} distance decreases by only 1 pm. The four distances between iron and the porphyrin nitrogens are similar in length (within 1 pm) and decrease by ~1 pm when the iron ion is oxidised. Thus, the changes in bond lengths are quite small, and it is therefore not very surprising that the inner-sphere reorganisation energy of this model is small, 8 kJ/mole. Interestingly, $_{ox}$ and $_{red}$ are of equal magnitude. From Figure 1, it can be seen that changes in the angles and dihedrals of the model upon oxidation are also very small. Similar small changes in geometry upon oxidation has also been observed by crystallography [57].

The second common combination of axial ligands is His-His. It is found in many *a*-, *b*-, and *c*-type cytochromes [3,56]. From Table 1, it can be seen that the Fe(porphine)$(Im)_2$ model behaves in a similar manner to the His-Met model: The Fe-N_{His} distance decrease by ~3 pm and the Fe-N_{Por} distances by 1 pm when the model is oxidised. Therefore, the reorganisation energy is also the same, 8 kJ/mole.

Cytochromes b_1 and b_{557} in bacterioferritin have yet another set of axial ligands: Met-Met [58-60]. Again, the result for this model is similar to those of the His-Met model, but the changes in the Fe-ligand distances are even smaller, all less than 1 pm. Therefore, this model has the lowest reorganisation energy of all investigated systems, 5 kJ/mole. This is an effect of the weaker Fe-S_{Met} bond, which is 40 pm longer than Fe-N_{His} bonds.

Recently, it was shown that in cytochrome *f*, the terminal amino group is a ligand to the haem group (in addition to a histidine group) [61]. It must then be deprotonated and neutral and was therefore modelled by CH_3NH_2. This is also a good model for a neutral lysine side chain, which has been suggested as a ligand in some cytochromes [3]. The amine group gave a slightly longer Fe-N

bond than histidine (205-208 pm), but the same change in bond length upon oxidation, and therefore the reorganisation energy is very similar to the His-His model, 9 kJ/mole.

The haem group in the d_1 domain of cytochrome cd_1 nitrite reductase has the axial ligands His and Tyr [62]. The tyrosine ligand is probably a deprotonated phenolate group and it has been modelled by $C_6H_5O^-$. The phenolate group binds at a shorter distance to iron than His, especially in the oxidised site (184 pm), and it changes by 15 pm during reduction. This large change in the Fe-O distance gives the model an appreciably larger reorganisation energy than for the other models, 47 kJ/mole. The large reorganisation energy is consistent with a low rate of electron transfer [63]. In fact, the phenolate group is protonated and dissociates from the haem group during catalysis in order to give place to the substrate [63]. Thus, this site has also a catalytic function.

Two other ligands have also been suggested to be present in electron-transfer sites (in addition to a histidine ligand), cysteine (Cys) and carboxylate (Asp or Glu) [3]. These ligands were modelled by CH_3SH^- and CH_3COO^-, and the results are similar to those of the His-Tyr model (Table 1). The charged ligand binds quite close to the iron ion (187-199 pm for the carboxylate group and 222-238 pm for the thiolate). This leads to an increase in the Fe-N distances, and also to a larger variation in the Fe-N_{Por} distances. The Fe-N_{His} distance for the cysteine complex is unexpectedly long (211-215 pm, compared to 202-207 pm in the other complexes). This indicates that the electronic structure of this complex is different from all the other complexes, with a significant transfer of charge from thiolate to iron (the Mulliken charge and spin density on iron are 0.20 and 0.15 *e* lower than for the His-Met complex) [64]. The oxidised form of this complex is also special in that it is the only one with a slightly non-planar porphyrin ring. Although the decrease in the distance to the charged ligand during reduction is larger for cysteine than for the carboxylate group, the reorganisation energy is larger for the carboxylate complex, 26 kJ/mole compared to 20 kJ/mole. This is probably owing to the longer and weaker bond to the thiolate group.

There are many haem proteins with other sets of ligands (His, Cys, Tyr, Asp, or Glu), typically with only five ligands or with a water molecule or substrate as the sixth ligand [3,56]. However, they are invariably catalytic or transport sites, rather than electron carriers. There are several

reasons why such sites are not used for electron transfer. First, our results show that charged ligands have a larger reorganisation energy than uncharged ligands. Second, the open coordination site is often occupied by a water molecule only in the oxidised state. Therefore, the water molecule shows a large change in geometry during the redox process and gives a large reorganisation energy. Third, five-coordinate complexes prefer the high-spin state, whereas six-coordinate complexes tend to be low spin. Therefore, there is a great risk for a spin crossing, which makes the reaction slower and give rise to larger reorganisation energies because of different bond lengths in the two spin states [65]. Fourth, the open coordination site also makes the complex sensitive to poisoning by small ligands, such as CO and NO, or binding of O_2, which can give rise to the formation of hazardous peroxides and superoxides.

In conclusion, our calculations give a quite complete picture of the effect of axial ligands in the cytochromes. The two commonly used ligand sets, His-Met and His-His, both give a very low reorganisation energy (around 8 kJ/mole). Therefore, they can be used interchangeably, and the choice is more determined by the reduction potential than by the reorganisation energy (Met gives a 170-300 mV higher potential than His) [3,66,67]. Interestingly, the Met-Met set of axial ligands gives the lowest reorganisation energy of all investigated complexes. The reason why this set of ligands is not more often used is probably that the Fe-S_{Met} interaction is so weak that the complex has a low stability, especially as the difference in reorganisation energy is not very large. Finally, we have seen that charged ligands give rise to about three times higher reorganisation energies than the uncharged ligands. This is most likely the reason why such ligands are rare in electron carriers but frequently used in catalytic sites (and it nicely illustrates the importance for electron-transfer proteins to have a low inner-sphere reorganisation energy).

Comparison with experimental data

Much experimental data is available for the structure of haem complexes with various axial ligands, both from studies of small inorganic models and from crystal structures of haem proteins. In Table 3 we have gathered some data with relation to the studied complexes. For the *b* and *c*-type cytochromes, a large number of structures have been published for the oxidised complexes.

Therefore, we have only listed the most accurate structures in the Brookhaven protein data bank (those with the lowest resolution). From the model complexes (which are most accurately determined), we can conclude that our calculated Fe-N_{Por}, Fe-N_{His}, Fe-S_{Met}, and Fe-S_{Cys} distances are all slightly too long, by 2-3, 4-5, 6, and 3 pm, respectively. This reflects a systematic error of the B3LYP method; similar errors were observed for iron-sulphur clusters and blue copper proteins [40,43,52]. However, it is also clear that the discrepancy is the same (within 1 pm) for the two oxidation states. Therefore, the change in the Fe-ligand bond lengths upon reduction is accurately reproduced in our models. Consequently, we can expect the calculated reorganisation energy to be quite reliable.

As regards the protein structures, the uncertainty in the metal-ligand distances are typically larger than in our calculated structures [68]. Therefore, only differences larger than about 10 pm can be expected to be significant, unless the average of several crystal structures is used. With this in mind, we again see the tendency of our calculations to give a too long Fe-S_{Met} bond, whereas the Fe-O_{Tyr} bond seems to be reasonable. However, for bacterioferritin, the crystal structure gives 24-29 pm *longer* Fe-S_{Met} bonds than in our calculations (and thus ~32 pm longer than in model complexes) [59]. This difference may be caused by the low resolution of the crystal structure (0.28 nm). Alternatively, interactions with the surrounding protein may be of the same strength as the weak Fe-S_{Met} interaction and favour longer bonds.

There is also much information about the reorganisation energy of haem proteins. In particular, the total self-exchange reorganisation energy of cytochrome *c* has been experimentally determined to 70-140 kJ/mole [69-71]. The self-exchange reorganisation energy for cytochrome b_5 is similar, 90-130 kJ/mole [69]. The outer-sphere contribution to the reorganisation energy has been calculated by various methods to 13-100 kJ/mole [15,17,22,24,31-33], compared to 100-160 kJ/mole for haem in water [15,22]. Thus, the inner-sphere reorganisation energy of the haem unit should be rather small, and it has been estimated to be 0-48 kJ/mole [15,17,22,32,33]. Our calculations indicate that it should be in the lower part of this range, around 8 kJ/mole, and they provide the first direct quantum mechanical estimate of this quantity (and therefore the most accurate estimate). This

comparison also shows that reorganisation energies are quite hard to estimate, both by theoretical and experimental methods.

How is the low reorganisation energy achieved

In order to investigate how cytochromes have achieved such a low reorganisation energy, we have optimised four model complexes, as is shown in Table 4. First, we studied the octahedral $Fe(H_2O)_6$ complex, as a model of an iron ion in water solution. It can be seen that already this complex has a rather modest reorganisation energy, 65 kJ/mole, although all Fe-O distances change by 10 pm upon reduction. This shows that an octahedral structure is favourable for electron transfer, since it does not lead to any changes in the angles of the complex, provided that the coordination number is preserved.

Second, we studied the $Fe(NH_3)_6$ complex. Here, the reorganisation energy is lower, 21 kJ/mole. The reason for this decrease is the smaller change in the Fe-N distances (5-8 pm) and the weaker bonds, especially in the oxidised state (as can be seen from the longer bond lengths). Thus, the use of ligands with soft bonds (nitrogen donors instead of oxygen) is a second mechanism used by the cytochromes to reduce the reorganisation energy.

Third, it is conceivable that the porphyrin ring may restrict the variation in the Fe-N_{Por} distances by covalent strain. Therefore, we optimised the structure of $Fe(NH(CH)_3NH)_2(Im)(S(CH_3)_2)$, where the porphyrin ring has been broken into two halves (c.f. Figure 2). This is appropriate for our purpose, because there is no longer any ring strain in this molecule, although it retains the double negative charge, the number of carbon bonds in each half-ring, and approximately the same ligand properties. Interestingly, this model shows appreciably larger changes in the equatorial Fe-N distances upon reduction (5-6 pm, similar to those of the ammonia complex) than the porphyrin model. The changes for the axial ligands are similar to those of the full porphyrin model. From this, we can conclude that the porphyrin ring elongate the Fe-N_{Por} distances, but more for Fe(III) (9 pm) than for Fe(II) (~3 pm). The reorganisation energy of this complex is twice as high as the

corresponding porphyrin model, 16 kJ/mole. Thus, covalent strain decreases the reorganisation energy for the haem group in the cytochromes by 8 kJ/mole.

Several authors have emphasised the low-spin state as a cause of the low reorganisation energy of the cytochromes [1,6,54]. We have quantified this suggestion by calculating the structures of the His-His cytochrome model at the high-spin state (shown in Table 4). These structures are 26-29 kJ/mole higher in energy, confirming the experimental observation that the low-spin structures are most stable. As expected, the high-spin structures exhibit appreciably longer Fe-ligand bonds (7 pm for Fe-N_{Por} and 21-31 pm for Fe-N_{His}) and an appreciably larger change in these distances upon oxidation (2 pm for Fe-N_{Por} and 13 pm for Fe-N_{His}). Therefore, the high-spin model has more than three times as high reorganisation energy as the low-spin model (28 kJ/mole), confirming the importance of a low-spin state.

Comparison with blue copper proteins and iron-sulphur clusters

In this article, we have shown that the inner-sphere reorganisation energy of cytochromes is very low if the axial ligands are not charged, 5-9 kJ/mole. This is appreciably lower than for the other electron-transfer proteins. With the same methods, we have estimated the inner-sphere reorganisation energy of the blue copper proteins, the binuclear Cu_A, and various iron-sulphur clusters with one to four iron atoms to 62-90, 43, and 40-75 kJ/mole, respectively [34,35,43]. For four types of blue copper proteins, rubredoxin, and [2Fe-2S] ferredoxin, we have also shown that the reorganisation energy is approximately halved in the protein (to 20-44 kJ/mole), owing to the differing dielectric properties of the protein and direct hydrogen bonds to the ligands [36,43]. It is most likely that the same is true for all blue-copper proteins and iron-sulphur clusters, whereas the inner-sphere reorganisation energy of the cytochromes is hardly changed since these sites have a low net charge and no hydrogen bonds directly to the iron-ligating atom. Still, the reorganisation energy of the cytochromes is 10-30 kJ/mole lower than for the other proteins. Thus, haem seems to be inherently better suited for electron transfer than the other two sites, at least in terms of the inner-sphere reorganisation energy.

The question then naturally arises as to why not only cytochromes are used as electron carriers in nature. Reasonable answers can be found directly from the Marcus equation (1). It contains two additional terms that determine the rate of electron transfer, the electronic coupling element and the reduction potential. For example, the other two sites involve cysteine ligands, which form metal bonds with appreciable charge delocalisation and therefore favourable electron-transfer paths. Moreover, the reduction potentials differ for the three types of sites as was discussed above. A third factor is the outer-sphere component of the reorganisation energy, which may vary quite a lot for various proteins (also within the groups of electron carriers). We currently study the importance of these factors for the rate of electron transfer in our laboratory.

Finally, it should be noted that the total reorganisation energy should not necessarily be minimised. Instead it should be matched to the driving force (the reduction potential difference) of the reaction. For many reactions the change in free energy during the reaction is low and then the reorganisation energy should be minimised. However, when this is not the case, a too low reorganisation energy would actually decrease the rate of electron transfer (8 kJ/mole corresponds to a potential difference of 0.08 V).

Other answers are also conceivable. For example, historical reasons may favour iron-sulphur clusters and disfavour copper sites. Moreover, porphyrins are oxidisable and it may be beneficial for an organism to have backup systems with alternative metals.

It is informative to compare how the three types of sites have achieved their low reorganisation energy. Some mechanism are used by all three sites. For example, they all employ N- and S-donor ligands but avoid oxygen atoms in the first coordination sphere. As we saw above, this is because oxygen forms stronger bonds, which give rise to a higher reorganisation energy. This has also been observed experimentally [72]. A methionine ligand is especially appropriate in this respect, giving very flexible bonds, which can change much at a small expense of energy. We have seen this for the cytochromes and even clearer for the blue copper proteins [35,37,40].

Second, all three electron-carrier sites employ delocalised systems, the cytochromes over the haem ring, and iron-sulphur clusters and blue copper proteins over metal-sulphur bonds. The effect

is especially pronounced in the polynuclear clusters. This seems to be an important property of the electron-transfer sites. It extends the site, thereby increasing the electronic coupling element between the donor and acceptor sites. It also makes it easier to hide the site from the solvent in the protein. Moreover, it gives a directionality of the site for electron transfer, i.e. it is easier to send the electron through delocalised bonds than in other directions [73,74]. This, together with stability considerations, may explain why bi- and polynuclear iron-sulphur clusters are more common than rubredoxin sites.

Two of the sites employ iron whereas the third uses copper. Which of the metals is most appropriate for electron transfer? Our results show that iron is inherently much better than copper, at least in terms of reorganisation energies [35,43]. This is because Fe(II) and Fe(III) prefer the same coordination number and the same geometry. Cu(I) and Cu(II), on the other hand, have distinctly different preferences both in coordination number and geometry. For example, as we saw in Table 4, the inner-sphere reorganisation energy of $Fe(H_2O)_6$ is 65 kJ/mole and the octahedral geometry of the complex is retained for both oxidation states. However, for the corresponding copper complex, Cu(II) is (distorted) octahedral, whereas Cu(I) prefers to become three coordinate. Naturally, this gives a very high reorganisation energy (336 kJ/mole) [35]. Yet, even if the complex is forced to be octahedral, the reorganisation energy is still twice as high as for iron (112 kJ/mole), owing to the Jahn-Teller distortion of Cu(II) and to the stronger bonds (higher force constants) of copper.

The same applies to $Fe(H_2O)_4$, which has almost the same reorganisation energy as $Fe(H_2O)_6$, 66 kJ/mole [43]. Again, Cu(I) prefers to have only three ligands in the corresponding copper complex, giving a reorganisation energy of 247 kJ/mole [35]. If Cu(I) is forced to have four ligands, the reorganisation energy is even higher than for the $Cu(H_2O)_6$ complex (186 kJ/mole), because Cu(II) assumes a square-planar structure, whereas Cu(I) becomes tetrahedral. Therefore, copper can only achieve a low reorganisation energy by the ingenious choice of ligands employed in the blue copper proteins (62 kJ/mole [35]), whereas already $Fe(H_2O)_6$ has a similar reorganisation energy (65 kJ/mole). If we use the same blue-copper ligand models for iron ($Fe(Im)_2(SCH_3)(S(CH_3)_2)$), we

get a site with a reorganisation energy of only 26 kJ/mole. As can be seen in Table 5, this is because all metal-ligand bond length changes are smaller than in the copper site. Thus, we predict that an iron-substituted blue copper protein would have a lower reorganisation energy than the native protein, provided that the coordination number does not change.

Finally, the three electron carriers also employ some mechanisms of their own to reduce the reorganisation energy. The blue copper proteins use a cysteine ligand to overcome the differences in the coordination number and geometric preferences. The thiolate group donates charge to Cu(II). This gives it partly Cu(I) character, thereby changing its preferred structure towards a tetrahedron [35]. For the binuclear Cu_A site in cytochrome *c* oxidase and nitrous oxide reductase, the delocalisation of charge between the two copper ions reduce the changes in metal-ligand bond lengths, and therefore lower the reorganisation energy [34,75].

A low coordination number was actually unfavourable for the blue copper site (owing to the differing coordination preferences of the two oxidation states) [35], whereas it is favourable for the iron-sulphur clusters. This is because the two oxidation states of iron both prefer a tetrahedral geometry with four cysteine ligands [43]. Moreover, there are fewer metal-ligand bonds in the four-coordinate site, which reduces the reorganisation energy, even if it is partly compensated for by a larger change in the bond length upon oxidation.

Finally, we have seen that the cytochromes reduce the reorganisation energy by choosing ligands that give rise to a low-spin site (by 20 kJ/mole; c.f. Table 4). Interestingly, each iron ion is in its high-spin state in the iron-sulphur clusters (although the polynuclear sites are typically antiferromagnetically coupled to a low total spin). Therefore, it may be instructive to investigate the reorganisation energy of a low-spin iron-sulphur site. This is done in Table 5 for the rubredoxin site ($Fe(SCH_3)_4^{2-/-}$; the low-spin state of these complex is 98-223 kJ/mole less stable than the high-spin state). It can be seen that for this site, the low-spin state actually gives a slightly larger reorganisation energy (54 kJ/mole compared to 40 kJ/mole for the high-spin state). As expected, the low-spin case gives shorter Fe-S bonds (by 6-13 pm). However, there is a larger variation in the bond lengths for the reduced low-spin site. Therefore, the change in bond lengths for the low-spin

site is smaller than for the high-spin site for two of the bonds (6-7 pm, compared to 10 pm), but larger for the other two bonds (12 pm). The higher reorganisation energy is also partly caused by the S-Fe-S angles, which in the reduced low-spin site show a much larger variation than in the high-spin site (94-142 compared to 109-110°). All these differences are caused by differences in the electronic structure of the two spin states.

As we saw above, the cytochromes also use covalent strain in the porphyrin ring to reduce the reorganisation energy by ~8 kJ/mole. This mechanism is most interesting, since it has been suggested that the blue copper proteins constrain the structure of the copper site, thereby reducing the reorganisation energy [3,13,76,77]. We have argued strongly against the suggestion that strain, in the sense of Warshel [55,78] (i.e. local distortions caused by covalent and repulsive Van der Waals interactions, like those in the porphyrin ring), plays any significant role for the function of these proteins [35,41]. In particular, our detailed calculations showed that covalent strain actually tends to increase the reorganisation energy of these sites, rather than decrease it [36]. It is therefore informative to compare the haem group and the blue copper proteins. The major difference between the two sites is that the porphyrin ring is held together by strong covalent bonds and is constrained by the aromaticity of the ring. In the protein, on the other hand, the local arrangement of ligands is determined weak torsional constraints and non-bonded interactions. Covalent bonds are stronger than metal-ligand bonds, whereas torsions and non-bonded interactions are weaker. Therefore, the iron ion is strained in the haem group, whereas it is more likely that the protein will distort if the preferences between the metal and the protein differ [55]. Similar conclusions have been reached by leading biophysical scientists regarding the role of covalent strain in enzyme catalysis [78,79,80].

In conclusion, we have in this paper studied the inner-sphere reorganisation energy of the three common types of electron carriers in nature. The results show the inherent suitability of the various sites for electron transfer. We have also discussed what mechanism the various sites have used to reduce the reorganisation energy. Together, all these results illustrate the ingenious construction of these biological systems and how similar problems can be solved in different ways.

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Legends to the Figures

Figure 1. The difference in geometry between the reduced and oxidised (shaded) forms of the cytochrome *c* model Fe(porphine)(imidazole)($S(CH_3)_2$).

Figure 2. The difference in geometry between the reduced and oxidised (shaded) forms of the cytochrome *c* model $Fe(NH(CH)_3NH)_2$(imidazole)($S(CH_3)_2$) with a broken porphyrin ring.

Table 1. Optimised geometries of seven cytochrome models. Amt is an uncharged amino terminal.

Axial ligands		Oxidation		Distance to Fe (pm)	
1	2	state	N_{Por}	Ligand 1	Ligand 2
Met	Met	II	202	240	240
		III	202	240	240
His	Met	II	202	203	243
		III	201	200	244
His	His	II	202	205	205
		III	201	202	203
His	Amt	II	202-203	203	208
		III	201-202	200	205
His	Cys	II	202	211	238
		III	201-203	215	222
His	Tyr	II	202-203	206	199
		III	201-203	207	184
His	Glu	II	202-203	205	199
		III	201-202	207	187

Table 2. Inner-sphere reorganisation energies for seven cytochrome models. Amt is an uncharged amino terminal.

Axial ligands		Reorganisation energy (kJ/mole)		
1	2	*red*	*ox*	*i*
Met	Met	2.7	2.1	4.8
His	Met	4.2	4.1	8.3
His	His	3.7	4.5	8.2
His	Amt	4.2	4.4	8.6
His	Cys	9.7	10.3	20.0
His	Tyr	21.2	25.8	47.0
His	Glu	13.0	13.4	26.4

Table 3. Experimental structures of porphyrin complexes with relevance to the present investigation. For proteins, the structure with the lowest resolution in the Brookhaven protein data bank has been used.

Axial ligands		Oxidation	Reference	Distance to Fe (pm)		
1	2	state		N_{Por}	Ligand 1	Ligand 2
Met	Met	III	[59]	201-202	264	269
SR_2	SR_2	II	[81]	200	234	234
SR_2	SR_2	III	[81]	198	233	235
His	Met	II	[82]	197-200	197	235
		III	[83]	197-202	203	236
His	His	III	[84]	197-200	200	208
Im	Im	II	[85]	200	201	201
		III	[86]	199-200	196	199
His	Amt	III	[61]	201-202	208	205
CO	RS^-	II	[65]			235
His	Tyr	III	[62]		198-202	185-189

Table 4. Geometries and inner-sphere reorganisation energies (kJ/mole) for some six-coordinate iron models. All complexes were studied in the high-spin state, except $Fe(NH(CH)_3NH)_2(Im)(S(CH_3)_2)$, which was considered to be low spin. L_{eq} represents the four equatorial ligands, whereas L_{ax1} and L_{ax2} are the axial ligands, imidazole and $S(CH_3)_2$, respectively, in the complexes with different ligands.

Model	Oxidation state	Reorg. energy	Distance to Fe (pm)		
			L_{eq}	L_{ax1}	L_{ax2}
$Fe(H_2O)_6$	II	30.5	215	215	215
	III	34.9	205	205	205
$Fe(NH_3)_6$	II	9.5	227-231	231	231
	III	11.2	222-223	223	223
$Fe(Por)(Im)_2$ high spin	II	11.8	208-209	236	237
	III	15.9	206-207	223	224
$Fe(Por)(Im)(S(CH_3)_2)$ high spin	II	14.2	208-210	220	394
	III	25.4	205-206	218	283
$Fe(NH(CH)_3NH)_2(Im)(S(CH_3)_2)$	II	7.3	198-199	202	244
	III	8.3	193	202	247

Table 5. Geometries and inner-sphere reorganisation energies (kJ/mole) for some four-coordinate models. All complexes were assumed to be high spin unless otherwise stated. The order of the L_1-L_4 ligands is the same as in the chemical formula.

Model	Oxidation state	λ_i kJ/mole	Distance to Fe (pm)			
			L_1	L_2	L_3	L_4
$Fe(Im)_2(SCH_3)(S(CH_3)_2)$	II	12.6	202	210	225	247
	III	13.3	203	204	224	246
$Cu(Im)_2(SCH_3)(S(CH_3)_2)$ [35]	I	32.7	214	215	232	237
	II	28.8	204	205	218	267
$Fe(SCH_3)_4$; low spin	II	28.1	229	229	235	236
	III	25.6	222	223	223	224
$Fe(SCH_3)_4$; high spin [43]	II	21.4	242	242	242	242
	III	18.3	232	232	232	232