A Comparison of the Inner-Sphere Reorganization Energies of Cytochromes, Iron−Sulfur Clusters, and Blue Copper Proteins

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Inner-sphere reorganization 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 reorganization energy is very low, 5−9 kJ/mol. If one of the axial ligands is charged, the reorganization energy is higher, 20−47 kJ/mol, but such sites are normally not used in electron carriers. The former reorganization energies are appreciably smaller than what was found for blue copper proteins (62−90 kJ/mol), the dimeric Cu_A site in cytochrome *c* oxidase and nitrous oxide reductase (43 kJ/mol), and six different types of iron-sulfur clusters with one, two, or four iron atoms (40−75 kJ/mol), 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 reorganization energy of the three commonly used electron carriers. All three types of sites have reduced the reorganization energy by using a delocalized 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 bonds weaker than those of copper. The low-spin state of the cytochrome has a ∼20 kJ/mol lower reorganization energy than that of 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 reorganization energy by 8 kJ/mol.

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-sulfur 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 with 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-sulfur clusters consist of iron ions surrounded by four sulfur ions, either cysteine thiolate groups or inorganic sulfide 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 sulfurs, 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 to the standard hydrogen electrode), and the iron-sulfur 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-sulfur clusters, the second complex involves one iron-sulfur cluster and several cytochromes, and the final complex (at the highest potential) contains two haem groups and two copper centers.[1]

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

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

Here, H_{DA} is the electronic coupling element, which is a function of the overlap of the wave functions of the two states involved in the reaction. It depends on the delocalization of the electron

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to be transferred in the metal site and the protein matrix between the two active sites. ΔG_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. λ is the reorganization 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 reorganization energy, depending on which atoms are relaxed. For a metal-containing protein, the inner-sphere reorganization energy is associated with the structural change of the first coordination sphere, whereas the outer-sphere reorganization energy involves structural changes of the remaining protein as well as the solvent.

Several groups have studied the reduction potential energy of cytochromes[16−24] or iron-sulfur 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 known, the results are worse.[20]

The outer-sphere reorganization energy of cytochromes has also been estimated by theoretical methods.[7,17,22,24,31−33] However, no direct estimate of the inner-sphere reorganization 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 reorganization energy is most interesting, since it is the only parameter in eq 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 centers 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 reorganization 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 reorganization energy of several types of iron-sulfur clusters, both in a vacuum and in the protein.[43] In this paper, we complete this investigations by studying the inner-sphere reorganization energy of a number of cytochrome models. This gives us the opportunity to contrast the various sites and to discuss how they have optimized their electron-transfer function.

Methods

Quantum chemical geometry optimizations 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-ζ 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 optimized until the change in energy between two iterations was below 10^{-6} Hartree (2.6 J/mol) and the norm of the internal gradients was below 10^{-3} a.u. (0.053 pm or 0.057°). No symmetry restraints were imposed.

TABLE 1: Optimized Geometries of Seven Cytochrome Models[a]

axial ligands			distance to Fe (pm)		
1	2	oxidation 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

[a] Amt is an uncharged amino terminal.

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 reorganization energy was estimated in the same way as for the blue copper proteins:[35] The reorganization energy for the oxidized complex (λ_{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 reorganization energy for the reduced complex (λ_{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 oxidized complex. This is actually the definition of the reorganization 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 eq 1 describes the relation between the total reorganization energy and the rate constants, which are measured experimentally. This relation is based on several approximations. The total inner-sphere reorganization energy for a self-exchange reaction (λ_i) is the sum of λ_{ox} and λ_{red}. In variance to the outer-sphere reorganization energy, the inner-sphere reorganization 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 Reorganization Energy of the Cytochrome Models. We have calculated geometries and inner-sphere reorganization energies of iron porphine with seven different sets of axial ligands. The results are collected in Tables 1 and 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

TABLE 2: Inner-Sphere Reorganization Energies for Seven Cytochrome Models[a]

axial ligands		reorganization energy (kJ/mol)		
1	2	λ_{red}	λ_{ox}	λ_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

[a] Amt is an uncharged amino terminal.

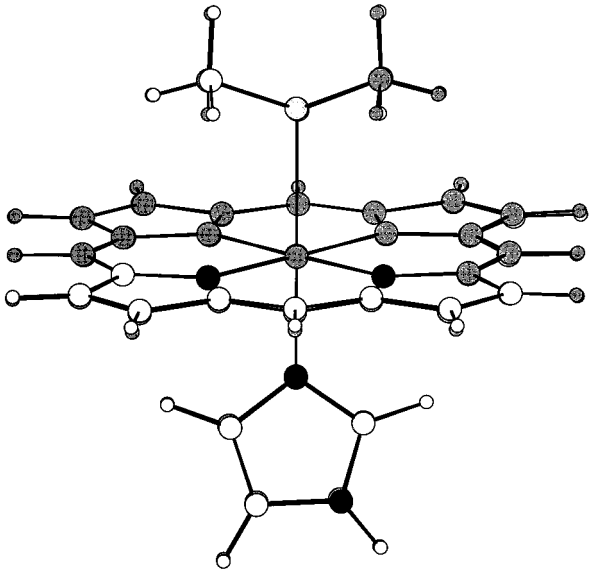

Figure 1. Difference in geometry between the reduced and oxidized (shaded) forms of the cytochrome *c* model Fe(porphine)(imidazole)-$(S(CH_3)_2)$.

in length (within 1 pm) and decrease by ~1 pm when the iron ion is oxidized. Thus, the changes in bond lengths are quite small, and it is therefore not very surprising that the inner-sphere reorganization energy of this model is small, 8 kJ/mol. Interestingly, λ_{ox} and λ_{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 manner similar to that of the His−Met model: The Fe−N_{His} distances decrease by ~3 pm and the Fe−N_{Por} distances by 1 pm when the model is oxidized. Therefore, the reorganization energy is also the same, 8 kJ/mol.

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 reorganization energy of all investigated systems, 5 kJ/mol. 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 modeled 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 that of histidine (205−208 pm) but the same change in bond length upon oxidation, and therefore, the reorganization energy is very similar to that of the His−His model, 9 kJ/mol.

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 modeled by $C_6H_5O^-$. The phenolate group binds at a shorter distance to iron than His, especially in the oxidized 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 reorganization energy than that for the other models, 47 kJ/mol. The large reorganization 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 also has 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 modeled 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 those for the His−Met complex).[64] The oxidized form of this complex is also special in that it is the only one with a slightly nonplanar porphyrin ring. Although the decrease in the distance to the charged ligand during reduction is larger for cysteine than for the carboxylate group, the reorganization energy is larger for the carboxylate complex, 26 kJ/mol compared to 20 kJ/mol. 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 reorganization energy than that of uncharged ligands. Second, the open coordination site is often occupied by a water molecule only in the oxidized state. Therefore, the water molecule shows a large change in geometry during the redox process which gives a large reorganization 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 gives rise to larger reorganization 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 reorganization energy (around 8 kJ/mol). Therefore,

TABLE 3: Experimental Structures of Porphyrin Complexes with Relevance to the Present Investigation[a]

axial ligands				distance to Fe (pm)		
1	2	oxidation state	ref	N_{Por}	ligand 1	ligand 2
Met	Met	III	59	201−202	264	269
SR_2	SR_2	II	81	200	234	234
		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

[a] For proteins, the structure with the lowest resolution in the Brookhaven Protein Data Bank has been used.

they can be used interchangeably, and the choice is more determined by the reduction potential than by the reorganization 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 reorganization energy of all investigated complexes. The reason 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 reorganization energy is not very large. Finally, we have seen that charged ligands give rise to reorganization energies about 3 times higher than those of the uncharged ligands. This is most likely the reason 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 reorganization 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 oxidized 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−sulfur 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 reorganization energy to be quite reliable.

As regards the protein structures, the uncertainty in the metal−ligand distances are typically larger than those 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 too long a 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 those 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 favor longer bonds.

TABLE 4: Geometries and Inner-Sphere Reorganization Energies (kJ/mol) for Some Six-Coordinate Iron Models[a]

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

[a] 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.

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

How the Low Reorganization Energy Is Achieved. To investigate how cytochromes have achieved such a low reorganization energy, we have optimized four model complexes, as 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 this complex already has a rather modest reorganization energy, 65 kJ/mol, although all Fe−O distances change by 10 pm upon reduction. This shows that an octahedral structure is favorable 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 reorganization energy is lower, 21 kJ/mol. The reason for this decrease is the smaller change in the Fe−N distances (5−8 pm) and the weaker bonds, especially in the oxidized 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 reorganization energy.

Third, it is conceivable that the porphyrin ring may restrict the variation in the Fe−N_{Por} distances by covalent strain. Therefore, we optimized the structure of $Fe(NH(CH)_3NH)_2(Im)(S(CH_3)_2)$, where the porphyrin ring has been broken into two halves (cf. 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

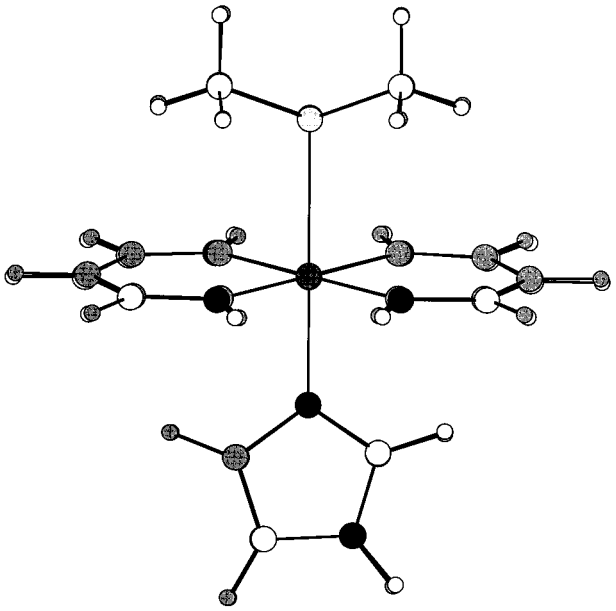

Figure 2. Difference in geometry between the reduced and oxidized (shaded) forms of the cytochrome *c* model $Fe(NH(CH)_3NH)_2$(imidazole)-$(S(CH_3)_2)$ with a broken porphyrin ring.

the equatorial Fe−N distances upon reduction (5−6 pm, similar to those of the ammonia complex) than those in 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 elongates the $Fe-N_{Por}$ distances, but more for Fe(III) (9 pm) than for Fe(II) (∼3 pm). The reorganization energy of this complex is twice as high as that of the corresponding porphyrin model, 16 kJ/mol. Thus, covalent strain decreases the reorganization energy for the haem group in the cytochromes by ∼8 kJ/mol.

Several authors have emphasized the low-spin state as a cause of the low reorganization 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/mol 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 a reorganization energy more than 3 times as high as that of the low-spin model (28 kJ/mol), confirming the importance of a low-spin state.

Comparison with Blue Copper Proteins and Iron−Sulfur Clusters. In this article, we have shown that the inner-sphere reorganization energy of cytochromes is very low if the axial ligands are not charged, 5−9 kJ/mol. This is appreciably lower than that for the other electron-transfer proteins. With the same methods, we have estimated the inner-sphere reorganization energy of the blue copper proteins, the binuclear Cu_A site ions, and various iron-sulfur clusters with one to four iron to 62−90, 43, and 40−75 kJ/mol, respectively.[34,35,43] For four types of blue copper proteins, rubredoxin, and [2Fe−2S] ferredoxin, we have also shown that the reorganization energy is approximately halved in the protein (to 20−44 kJ/mol), 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-sulfur clusters, whereas the inner-sphere reorganization 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 reorganization energy of the cytochromes is 10−30 kJ/mol lower than that 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 reorganization 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 eq 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 delocalization and therefore favorable 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 reorganization 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 reorganization energy should not necessarily be minimized. 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 reorganization energy should be minimized. However, when this is not the case, too low a reorganization energy would actually decrease the rate of electron transfer (8 kJ/mol corresponds to a potential difference of 0.08 V).

Other answers are also conceivable. For example, historical reasons may favor iron−sulfur clusters and disfavor copper sites. Moreover, porphyrins are oxidizable, 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 reorganization 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 reorganization 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 delocalized systems, the cytochromes over the haem ring and iron-sulfur clusters and blue copper proteins over metal−sulfur 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 delocalized bonds than in other directions.[73,74] This, together with stability considerations, may explain why bi- and polynuclear iron−sulfur 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 reorganization 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

TABLE 5: Geometries and Inner-Sphere Reorganization Energies (kJ/mol) for Some Four-Coordinate Models[a]

model	oxidation state	λ_i (kJ/mol)	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

[a] All complexes were assumed to be high spin unless otherwise stated. The order of the L_1-L_4 ligands is the same as that in the chemical formula.

distinctly different preferences both in coordination number and geometry. For example, as we saw in Table 4, the inner-sphere reorganization energy of $Fe(H_2O)_6$ is 65 kJ/mol, 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 reorganization energy (336 kJ/mol).[35] Yet even if the complex is forced to be octahedral, the reorganization energy is still twice as high as that for iron (112 kJ/mol), 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 reorganization energy as that for $Fe(H_2O)_6$, 66 kJ/mol.[43] Again, Cu(I) prefers to have only three ligands in the corresponding copper complex, giving a reorganization energy of 247 kJ/mol.[35] If Cu(I) is forced to have four ligands, the reorganization energy is even higher than that for the $Cu(H_2O)_6$ complex (186 kJ/mol) because Cu(II) assumes a square-planar structure whereas Cu(I) becomes tetrahedral. Therefore, copper can only achieve a low reorganization energy by the ingenious choice of ligands employed in the blue copper proteins (62 kJ/mol[35]), whereas $Fe(H_2O)_6$ already has a similar reorganization energy (65 kJ/mol). 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 reorganization energy of only 26 kJ/mol. 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 reorganization 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 reorganization 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 toward a tetrahedron.[35] For the binuclear Cu_A site in cytochrome *c* oxidase and nitrous oxide reductase, the delocalization of charge between the two copper ions reduces the changes in metal−ligand bond lengths, and therefore lowers the reorganization energy.[34,75]

A low coordination number was actually unfavorable for copper sites (owing to the differing coordination preferences of the two oxidation states),[35] whereas it is favorable for the iron−sulfur 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 reorganization 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 reorganization energy by choosing ligands that give rise to a low-spin site (by 20 kJ/mol; cf. Table 4). Interestingly, each iron ion is in its high-spin state in the iron−sulfur clusters (although the polynuclear sites are typically antiferromagnetically coupled to a low total spin). Therefore, it may be instructive to investigate the reorganization energy of a low-spin iron−sulfur site. This is done in Table 5 for the rubredoxin site ($Fe(SCH_3)_4^{2-/-}$; the low-spin state of these complexes is 98−223 kJ/mol less stable than the high-spin state). It can be seen that for this site, the low-spin state actually gives a slightly larger reorganization energy (54 kJ/mol compared to 40 kJ/mol 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 that for the high-spin site for two of the bonds (6−7 pm, compared to 10 pm) but larger than that for the high-spin site for the other two bonds (12 pm). The higher reorganization 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 reorganization energy by ∼8 kJ/mol. 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 reorganization 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 calculations in the protein showed that covalent strain actually tends to increase the reorganization 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 by weak torsional constraints and nonbonded interactions. Covalent bonds are stronger than metal−ligand bonds, whereas torsions and nonbonded 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 reorganization 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 reorganization 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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References and Notes

(1) Cowan, J. A. *Inorganic Biochemistry, an Introduction*; Wiley-VCH: New York, 1997.
(2) Palmer, G.; Reedijk, J. *Eur. J. Biochem.* **1991**, *200*, 599.
(3) Fraústo da Silvia, J. J. R.; Williams, R. P. J. *The Biological Chemistry of the Elements*; Clarendon Press: Oxford, 1994.
(4) Beinert, H. *Science* **1997**, *277*, 653.
(5) Cammack, R. *Adv. Inorg. Chem.* **1992**, *38*, 281−322.
(6) Holm, R. H.; Kennepohl, P.; Solomon, E. I. *Chem. Rev.* **1996**, *96*, 2239−2314.
(7) Matsubara, H.; Saeki, K. *Adv. Inorg. Chem.* **1992**, *38*, 223.
(8) Adman, T. *Adv. Protein Chem.* **1991**, *42*, 145−197.
(9) Messerschmidt, A. *Struct. Bonding (Berlin)* **1998**, *90*, 37−68.
(10) Sykes, A. G. *Adv. Inorg. Chem.* **1990**, *36*, 377−408.
(11) Beinert, H. *Eur. J. Biochem.* **1997**, *245*, 521.
(12) Kroneck, P. M. H.; Antholine, W. E.; Kastrau, D. H. W.; Buse, G.; Steffens, G. C. M.; Zumft, W. G. *FEBS Lett* **1990**, *268*, 274.
(13) Gray, H. B.; Malmström, B. G.; Williams, R. J. P. *J. Biol. Inorg. Chem.* **2000**, *5*, 551−559.
(14) Zhou, H.-X. *J. Biol. Inorg. Chem.* **1997**, *2*, 109.
(15) Marcus, R. A.; Sutin, N. *Biochim. Biophys. Acta* **1985**, *811*, 265.
(16) Churg, A. K.; Warshel, A. *Biochemistry* **1986**, *25*, 1675.
(17) Churg, A. K.; Weiss, R. M.; Warshel, A.; Takano, T. *J. Phys. Chem.* **1983**, *87*, 1683.
(18) Gunner, M. R.; Honig, B. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 9151.
(19) Gunner, M. R.; Alexov, E.; Torres, E.; Lipovaca, S. *J. Inorg. Biol. Chem.* **1997**, *2*, 126.
(20) Langen, R.; Warshel, A. *J. Mol. Biol.* **1992**, *224*, 589.
(21) Martel, P. J. *J. Biol. Inorg. Chem.* **1999**, *4*. 73.
(22) Muegge, I.; Qi, P. X.; Wand, A. J.; Chu, Z. T.; Warshel, A. *J. Phys. Chem. B* **1997**, *101*, 825.
(23) Rodgers, K. K.; S. G. Sligar *J. Am. Chem. Soc.* **1991**, *113*, 9419.
(24) Sharp, K. A. *Biophys. J.* **1998**, *73*, 1241.
(25) Jensen, G. M.; Warshel, A.; Stephens, P. J. *Biochemistry* **1994**, *33*, 10911.
(26) Mouesca, J.-M.; Chen, J. L.; Noodleman, L.; Bashford, D.; Case, D. A. *J. Am. Chem. Soc.* **1994**, *116*, 11898.
(27) Noodleman, L. *J. Biol. Inorg. Chem.* **1996**, *1*, 177.
(28) Stephens, P. J.; Jollie, D. R.; Warshel, A. *Chem. Rev.* **1996**, *96*, 2491.
(29) Swartz, P. D.; Ichiye, T. *Biophys. J.* **1997**, *73*, 2733.
(30) Swartz, P. D.; Beck, B. W.; Ichiye, T. *Biophys. J.* **1996**, *71*, 2958.
(31) Andrew, S. M.; Thomasson, K. A.; Northrup, S. H. *J. Am. Chem. Soc.* **1993**, *115*, 5516−5521.
(32) Zheng, C.; McCammon, J. A.; Wolynes, P. G. *Chem. Phys.* **1991**, *158*, 261.
(33) Zhou, H.-X. *J. Am. Chem. Soc.* **1994**, *116*, 10362.
(34) Olsson, M. H. M.; Ryde, U. *J. Am. Chem. Soc.*, in press.
(35) Olsson, M. H. M.; Ryde, U.; Roos, B. O. *Protein Sci.* **1998**, *7*, 2659−2668.
(36) Ryde, U.; Olsson, M. H. M. *Intern. J. Quantum Chem.* **2001**, *81*, 335−347.
(37) Olsson, M. H. M.; Ryde, U. *J. Biol. Inorg. Chem.* **1999**, *4*, 654−663.
(38) Olsson, M. H. M.; Ryde, U.; Roos, B. O.; Pierloot, K. *J. Biol. Inorg. Chem.* **1998**, *3*, 109−125.
(39) Pierloot, K.; De Kerpel, J. O. A.; Ryde, U.; Olsson, M. H. M.; Roos, B. O. *J. Am. Chem. Soc.* **1998**, *120*, 13156−13166.
(40) Ryde, U.; Olsson, M. H. M.; Pierloot, K.; Roos, B. O. *J. Mol. Biol.* **1996**, *261*, 586−596
(41) Ryde, U.; Olsson, M. H. M.; Roos, B. O.; De Kerpel, J. O. A.; Pierloot, K. *J. Biol. Inorg. Chem.* **2000**, *5*, 565−574.
(42) Ryde, U.; Olsson, M. H. M.; Pierloot, K. The Structure and Function of Blue Copper Proteins. In *Theoretical and Computational Chemistry*; L. A. Eriksson, Ed.; Elsevier: Amsterdam, 2001; Vol. 9, pp 1−56.
(43) Sigfridsson, E.; Olsson, M. H. M.; Ryde, U. *Inorg. Chem.* **2001**, *40*, 2509−2519.
(44) Hertwig, R. H.; Koch, W. *Chem. Phys. Lett.* **1997**, *268*, 345−351.
(45) Treutler D.; Ahlrichs R. *J. Chem. Phys.* **1995**, *102*, 346.
(46) Holthausen, M. C.; Mohr, M.; Koch, W. *Chem. Phys. Lett.* **1995**, *240*, 245−252.
(47) Ricca, A.; Bauschlicher, C. W. *J. Phys. Chem.* **1994**, *98*, 12899−12903.
(48) Ricca, A.; Bauschlicher, C. W. *Theor. Chim. Acta* **1995**, *92*, 123−131.
(49) Bauschlicher, C. W. *Chem. Phys. Lett.* **1995**, *246*, 40.
(50) Schäfer, A.; Huber, C.; Ahlrichs, R. *J. Chem. Phys* **1994**, *100*, 5829.
(51) Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley-Interscience: New York, 1986.
(52) Ryde, U.; Olsson, M. H. M.; Roos, B. O.; Carlos Borin, A. *Theor. Chem. Acc.* **2001**, *105*, 452−462.
(53) Sigfridsson, E.; Ryde, U. Manuscript in preparation.
(54) Lippard, S. J.; Berg, J. M. *Principles of Bioinorganic Chemistry*; University Science Books: Mill Valley, 1994.
(55) Ryde, U. In *Recent Res. Dev. Protein Eng.*, Research Signpost, Trivandrum.
(56) The Prosthetic Broups and Metalions in Protein Active Sites Database, Version 2 (http://bmbsgi11.leeds.ac.uk/promise, accessed Sept 2000).
(57) Takano, T.; Dickerson, R. E. *J. Mol. Biol.* **1981**, *153*, 95.
(58) Cheesman, M. R.; Thomson, A. J.; Greenwood, C.; Moore, G. R.; Kadir, F. *Nature* **1990**, *346*, 771.
(59) Frolow, F.; Smith, A. J.; Guest, J. R.; Harrison, P. M. *Nat. Struct. Biol.* **1994**, *1*, 453.
(60) George, G. N.; Richards, T.; Bare, R. E.; Gea, Y.; Prince, R. C.; Stiefel, E. I.; Watt, G. D. *J. Am. Chem. Soc.* **1993**, *115*, 7716.
(61) Carrell, C. J.; Schlarb, B. G.; Bendall, D. S.; Howe, C. J.; Cramer, W. A.; Smith, J. L. *Biochemistry* **1999**, *38*, 9590.
(62) Baker, S. C.; Saunders, N. F. W.; Willis, A. C.; Ferguson, S. J.; Hajdu, J.; Fülöp, V. *J. Mol. Biol.* **1997**, *269*, 440.
(63) Williams, P. A.; Fulöp V.; Garman, E. F.; Saunders, N. F. W.; Ferguson, S. J.; Hajdu, J. *Nature* **1997**, *389*, 406−412.
(64) Liu, H. I.;. Sono, M.; Kadkhodayan, S.; Hager, L. P.; Hedman, B.; Hodgson, K. O.; Dawson, J. H. *J. Biol. Chem.* **1995**, *270*, 10544−10550.
(65) Scheidt, W. R.; Reed, C. A. *Chem. Rev.* **1981**, *81*, 543.
(66) Raphael, A. L.; Gray, H. B. *J. Am. Chem. Soc.* **1991**, *113*, 1038.
(67) Tezcan, F. A.; Winkler, J. R.; Gray, H. B. *J. Am. Chem. Soc.* **1998**, *120*, 13383.
(68) Cruickshank, D. W. *Acta Crystallogr.* **1999**, *D55*, 583.
(69) Dixon, D. W.; Hong, X.; Woehler, S. E.; Mauk, A. G.; Sishta, B. P. *J. Am. Chem. Soc.* **1990**, *112*, 1082.
(70) Gupta, R. K. *Biochim. Biophys. Acta* **1973**, *292*, 291.
(71) Nocera, D. G.; Winkler, J. R.; Yocom, K. M.; Bordignon, E.; Gray, H. B. *J. Am. Chem. Soc.* **1984**, *106*, 5145.
(72) LeCloux, D. D.; Barrios, A. M.; Mizoguchi, T. J.; Lippard S. J. *J. Am. Chem. Soc.* **1998**, *120*, 9001−9014.
(73) Lowery, M. D.; Guckert, J. A.; Gebhard, M. S.; Solomon, E. I. *J. Am. Chem. Soc.* **1993**, *115*, 3012.
(74) Randall, D. W.; Gamelin, D. R.; LaCroix, L. B.; Solomon, E. I. *J. Biol. Inorg. Chem.* **2000**, *5*, 16−29.
(75) Karpefors, M.; Slutter, C. E.; Fee, J. A.; Aasa, R.; Källebring, B.; Larsson, S.; Vänngård, T. *Biophys. J.* **1996**, *71*, 2823.
(76) Malmström, B. G. *Eur. J. Biochem.* **1994**, *223*, 711−718.
(77) Williams, R. J. P. *Eur. J. Biochem.* **1995**, *234*, 363−381.
(78) Warshel, A. *Computer Modelling of Chemical Reactions in Enzymes and Solutions*; Wiley: New York, 1991; pp 155−158, 209−211.
(79) Levitt, M. In *Peptides, Polypeptides and Proteins*; Blout, E. R., Bovey, F. A., Goodman, M., Lotan, N., Eds.; Wiley: New York, 1974; pp 99−102.
(80) Fersht, A. *Enzyme Structure and Mechanisms*; W. H. Freeman, Co: New York, 1985; pp 341−342.
(81) Mashiko, T.; Marchon, J.-C.; Musser, D. T.; Reed, C. A.; Kastner, M. E.; Scheidt, W. R. *J. Am. Chem. Soc.* **1979**, *101*, 3653.
(82) Louie, G. V.; Brayer, G. D. *J. Mol. Biol.* **1989**, *210*, 313.
(83) Frazao, C.; Soares, C. M.; Carrondo, M. A.; Pohl, E.; Dauter, Z.; Wilson, K. S.; Hervas, M.; Navarro, J. A.; de la Rosa, M. A.; Sheldrick, G. M. Brookhaven Protein Data Bank Structure 1ctj.
(84) Mathews, F. S.; Czerwinski, E. W. *The Enzymes of Biological Membranes*; Plenum Press: New York, 1985; Vol. 4, p 235.
(85) Scheidt, W. R. *Acc. Chem. Res.* **1977**, *10*, 339.
(86) Collins, D. M.; Countryman, R.; Hoard, J. L. *J. Am. Chem. Soc.* **1972**, *94*, 2066.