JBIC (1997) 2:387–392

COMMENTARY

Ole Farver · Israel Pecht

The role of the medium in long-range electron transfer

Received: 2 January 1997 / Accepted: 6 February 1997

Abstract The role of the polypeptide matrix in electron transfer processes in proteins has been studied in two distinct systems: first in a protein where the induced ET is artificial, and second as part of the catalytic cycle of an enzyme. Azurins are structurally well-characterized blue single-copper proteins consisting of a rigid β-sheet polypeptide matrix. We have determined rate constants and activation parameters for intramolecular long-range electron transfer between the disulfide radical anions (generated by pulse radiolysis) and the copper(II) centre as a function of driving force and nature of the intervening medium in a large number of wild-type and single-site-mutated proteins. In ascorbate oxidase, for which the three-dimensional structure is equally well characterized, the internal ET from the type-I Cu(I) to the trinuclear Cu(II) centre has been studied. We find that the results correlate well with distance through well-defined pathways using a through-bond electron tunnelling mechanism.

Key words Ascorbate oxidase · Azurin · Copper protein · Electron tunnelling · Pathway calculation

It is the honour of God to conceal a thing; and the honour of kings to resolve it.
Proverbs: 25,2

O. Farver (✉)
Institute of Analytical Chemistry,
Royal Danish School of Pharmacy,
2, Universitetsparken,
DK-2100 Copenhagen Ø, Denmark
Tel. +45-35-37-08-50; Fax: +45-35-37-57-44;
e-mail: of@charon.dfh.dk

I. Pecht
Department of Immunology,
The Weizmann Institute of Science,
Rehovot 76100, Israel
Tel.:+ 972-89-34-40-20; Fax:+ 972-89-46-52-64;
e-mail: lipecht@weizmann.weizmann.ac.il

Introduction

The central subject in studies of long-range electron transfer (LRET) in proteins has been how distance and structure of the medium separating donor and acceptor influence electron tunnelling rates. Interpretation of experimental results in terms of the detailed chemical nature of the polypeptide matrix is a topic of the present debate, and pertinent questions are whether the shortest direct ET path is the one employed or whether structural and electronic properties of the medium provide more facile, albeit longer, ET routes [1–5].

We have investigated intramolecular LRET in azurins, which are rigid β-sheet structured blue, single-copper proteins [6–12] for many of which high-resolution structures have been determined [13–17]. Azurins contain two potential redox centres; hence there is no need for introducing external redox groups. Moreover, the copper ion is coordinated directly to amino acid residues, and a disulfide group is present at the opposite end of the molecule at a direct distance of 2.65 nm [13]. Significantly, the role of the disulfide bridge is most probably only structural, and the redox function of the protein is confined to the copper-containing domain. Therefore, the medium separating these two redox centers provides an appropriate system for introducing specific structural changes and correlating them with ET reactivity. As the distance between the electron donor and electron acceptor sites is most probably maintained constant in all azurins studied, these results provide a very useful basis for analysing the dependence of the internal ET rate constants on specific structural changes introduced in the separating medium which has not been selected for performing ET.

In contrast, we have also studied intramolecular ET in ascorbate oxidase between the electron uptake site and the centre where the oxidising substrate, O_2, binds and gets reduced to H_2O, i.e. a process which is part of the catalytic cycle of the enzyme [18, 19]. In spite of the short peptide stretch connecting the two centres, the

rate of ET is relatively slow because the driving force is close to zero. Thus, gating by nuclear rearrangements and substrate binding is important in this system.

ET in blue copper proteins

LRET between the two redox centres in azurin can be induced by reducing the disulfide group to and $RSSR^-$ radical anion by pulse radiolysis. This is then followed by an intramolecular ET reaction:

$$Az[Cu(II)RSSR^-] \rightarrow Az[Cu(I)RSSR]$$

We have determined rate constants of this intramolecular ET as a function of temperature an pH for a large number of wild-type (WT) and single-site-mutated azurins [6–12]. Specific structural changes were introduced into azurins, and their effects on LRET rates and activation parameters were analysed. Rate constants of the intramolecular ET at pH 7 and 298 K together with their activation parameters are summarized in Table 1.

The semiclassical Marcus theory for non-adiabatic processes predicts that intramolecular ET in proteins is governed by the standard free energy of reaction (ΔG^0), the nuclear reorganization energy (λ), and the electronic coupling (H_{DA}) between electron donor (D) and acceptor (A) at the transition state [20]:

$$k = \frac{2\pi}{\hbar} \frac{H_{DA}^2}{(4\pi\lambda RT)^{1/2}} e^{-(\Delta G^0+\lambda)^2/4\lambda RT} \quad (1)$$

The electronic coupling energy, H_{DA}, is expected to decay exponentially with the distance between D and A as:

$$H_{DA} = H_{DA}^0 \; e^{-\frac{\beta}{2}(r-r_0)} \quad (2)$$

The distance separating A and D in proteins may be considerable (≥ 1.0 nm), leading to a very small electronic coupling. Still, intramolecular ET over distances of 2.0 nm or more has been observed [21].

The activation enthalpy is given by the following relation [20]:

$$\Delta H^{\#} = \frac{\lambda}{4} + \frac{\Delta H^0}{2}\left(1 + \frac{\Delta G^0}{\lambda}\right) - \frac{(\Delta G^0)^2}{4\lambda} \quad (3)$$

For most azurins studied, $\Delta H^{\#}$ is constant within experimental error (cf. Table 1), a finding which supports our previous assumption that as long as mutations are not introduced at or near the copper site or the disulfide bond, the reorganization energy does not change significantly in this system.

The activation entropy includes a contribution from the distance dependence of the electronic coupling [20] (cf Eq. 2):

$$\Delta S^{\#} = \Delta S^* - R\beta(r-r_0) \quad (4)$$

Table 1 Kinetic and thermodynamic data for the intramolecular reduction of Cu(II)

Azurin	k_{298} (s^{-1})	E' (mV)	$-\Delta G^0$ (kJ mol^{-1})	$\Delta H^{\neq}$ (kJ mol^{-1})	$\Delta S^{\neq}$ (J K^{-1} mol^{-1})
Wild type					
Pseudomonas aeruginosa[a]	44 ± 7	304	68.9	47.5 ± 2.2	− 56.5 ± 3.5
Pseudomonas fluorescens[b]	22 ± 3	347	73.0	36.3 ± 1.2	− 97.7 ± 5.0
Alcaligenes species[a]	28 ± 1.5	260	64.6	16.7 ± 1.5	−171 ± 18
Alcaligenes faecalis[b]	11 ± 2	266	65.2	54.5 ± 1.4	− 43.9 ± 9.5
Mutant					
D23A[e]	15 ± 3	311	69.6	47.8 ± 1.4	− 61.4 ± 6.3
F110S[f]	38 ± 10	314	69.9	55.5 ± 5.0	− 28.7 ± 4.5
F114A[c]	72 ± 14	358	74.1	52.1 ± 1.3	− 36.1 ± 8.2
H35Q[d]	53 ± 11	268	65.4	37.3 ± 1.3	− 86.5 ± 5.8
I7S[f]	42 ± 8	301	68.6	56.6 ± 4.1	− 21.5 ± 4.2
M44K[d]	134 ± 12	370	75.3	47.2 ± 0.7	− 46.4 ± 4.4
M64E[f]	55 ± 8	278	66.4	46.3 ± 6.2	− 56.2 ± 7.2
M121L[c]	38 ± 7	412	79.3	45.2 ± 1.3	− 61.5 ± 7.2
V31W[g]	285 ± 18	301	68.6	47.2 ± 2.4	− 39.7 ± 2.5
W48A[g]	35 ± 7	301	68.6	46.3 ± 5.9	− 58.3 ± 6.0
W48F[g]	80 ± 5	304	68.9	43.7 ± 6.7	− 61.9 ± 9.7
W48S[g]	50 ± 5	314	69.9	49.8 ± 4.9	− 44.0 ± 3.5
W48Y[g]	85 ± 5	323	70.7	52.6 ± 6.9	− 30.2 ± 3.6
W48L[c]	40 ± 4	323	70.7	48.3 ± 0.9	− 51.5 ± 5.7
W48M[c]	33 ± 5	312	69.7	48.4 ± 1.3	− 50.9 ± 7.4

[a] From [6]
[b] From [7]
[c] From [9]
[d] From [8]
[e] From [11]
[f] From [10]
[g] From [12]

and ΔS^* is related to the standard entropy of reaction, ΔS^0:

$$\Delta S^* = \frac{\Delta S^0}{2}\left(1 + \frac{\Delta G^0}{\lambda}\right) \quad (5)$$

Using the experimentally determined rate constants and activation parameters of 19 different WT and mutated azurins (Table 1), and taking advantage of the above notion that the reorganization energy is most probably unaffected, we calculate $\lambda = 99.4 \pm 5$ kJ mol^{-1} and $\beta(r-r_0) = 24.6 \pm 1.2$.

Dutton and co-workers [2, 3] have analysed data derived from a large body of intramolecular electron transfer reactions and found that the free-energy-optimized rate constants for these processes correlate well with the edge-to-edge distance between donor and acceptor, with a decay factor $\beta = 14$ nm^{-1}. From the refined (0.19 nm) *Pseudomonas aeruginosa* azurin structure [13] the distance $(r-r_0)$ separating S_γ of Cys3 in the disulfide bridge and the copper-ligating S_γ, of Cys112, which represents the shortest edge-to-edge distance between electron donor and acceptor, is 2.51 nm. This yields a $\beta = 9.8 \pm 0.8$ nm^{-1}. The difference between the above β values is too large to be accounted for in terms of experimental error. Moreover, the calculated maximum LRET rate constant for azurin (i.e. for $\lambda = -\Delta G^0$) is 120 s^{-1}, whereas, using the correlation line of Farid et al. [3], the rate constant should be at least two orders of magnitude smaller. Distances for azurins (and ascorbate oxidase) are available from high-resolution structures, and no cofactors or other extraneous components are involved. In contrast, a considerable number of cases examined in the studies of Dutton et al. [2, 3] are dominated by cofactors, and in fact hardly any protein is present between the ET partners. Rather, the protein only provides the surrounding matrix, which is obviously having its impact, yet not in the context addressed in the present deliberations. Our edge-to-edge distance decay constant, $\beta = 9.8$ nm^{-1}, is in excellent agreement with that predicted for coupling decay along a strand of a β-sheet [22], and experimentally verified by Gray and co-workers [23] for ruthenium-modified cytochrome *c* and azurin. Obviously, the medium plays an important role in intramolecular protein LRET.

A theoretical approach addressing the issue of medium dependence of protein ET has been introduced by Beratan and Onuchic [24, 25]. We have employed their pathway model for identifying potential ET routes using the available high-resolution three-dimensional structures of *Ps. aeruginosa* azurin and its mutants [13–17]. For other mutants, structures based on 2D NMR studies and energy minimization calculations were employed. Pathway calculations suggest that the same two dominant electron transfer routes, shown in Fig. 1, operate in all azurins examined [6–12]: One path goes through the peptide chain to the copper-ligating imidazole of His46, while a more direct one leads through the polypeptide matrix via the buried indole ring of Trp48 to the copper-coordinating S_γ of Cys112. The electronic coupling factors were found to be $\Pi = 2.5 \times 10^{-7}$ and 4.7×10^{-8}, respectively.

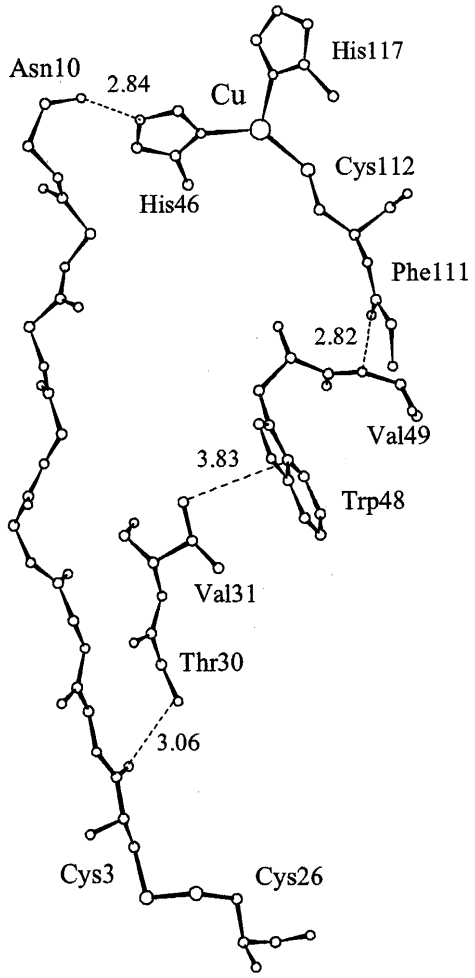

Fig. 1 Calculated pathways for ET from the sulfur of Cys3 to the copper centre in WT *Pseudomonas aeruginosa* azurin. Some interconnecting distances (three H-bonds and one van der Waals contact) are given (in Å). In the V31W mutant the closest distance between the two tryptophans (3.5 Å) occurs between W48 $C_{\zeta 3}$ and W31 C_{β}. The coordinates were taken from the Brookhaven Protein Databank [13]

Calculating effective covalent tunnelling lengths as defined in [26] in azurin, including hydrogen bonds and through-space contacts, yields $\sigma_1 = 4.07$ nm for the "His46 pathway" (27 covalent bonds and 1 H-bond), while σ_1 becomes 4.62 nm for the "Trp48 pathway" (19 covalent bonds, 2 H-bonds and 1 van der Waals contact). *All distances reported here for the rigid azurins are defined as the distance between S_γ of Cys3 and the atom coordinating to the copper ion*. For the former route we can now estimate a value for the distance decay constant, β, of 6.0 ± 0.4 nm^{-1}. This is in good agreement with theoretical calculations for tunnelling through a saturated aliphatic $(CH_2)_n$ chain ($\beta = 6.5$ nm^{-1}) [27] and also, in accord with results of Gray and coworkers

[22, 26], in studies of Ru-modified azurins (7.3 nm^{-1}, based on the σ_l, covalent path), although distance comparisons are difficult since the latter systems often involve different donors and/or acceptors. Hence, defining an unambiguous distance measure is problematic, as for instance are the so-called edge-to-edge distances.

Sykes et al. [28, 29] have studied ET in two His59-ruthenated algal plastocyanins and have found very small rate constants for intramolecular ET from Ru(II) to Cu(II) [$< 0.08\ s^{-1}$ (*Anabaena variabilis*) and $< 0.3\ s^{-1}$ (*Scenedesmus obliquus*)] in spite of a relatively short edge-to-edge distance of 1.19 nm and considerable driving forces of 25.1 and 28.0 kJ mol^{-1}, respectively. The shortest pathway from donor (His59) to acceptor (Cys84) calculated on the basis of the refined X-ray structure for poplar plastocyanin [30] consists of 38 covalent bonds and one extended H-bond (0.35 nm) between Glu60($O_{\ 1}$) and Asn76($N_{\delta 2}$). This would correspond to an effective covalent tunnelling length, σ_l, of no less than 5.93 nm. Thus, clearly, only the pathway model is capable of rationalizing the discrepancy between the observed very slow rates and the short direct separation distance.

Comparing the covalent tunnelling lengths for the above two pathways in azurin (or, equivalently, the two electronic coupling factors) the "Trp48 pathway" seems to be rather inferior. However, electronic interactions between the Cu(II) ion and its ligands were not included in the pathway analysis. Recently it has been proposed that a high degree of anisotropic covalency in the blue single-copper protein, plastocyanin, would enhance ET through the Cys ligand [31, 32]. By similar arguments, from the Ψ_{HOMO} ligand coefficients in azurin [33] it can be estimated that ET rates through Cys would be increased to ~ 150 times that of ET via one of the His ligands. This means that the "Trp48" and "His 46" pathways would be about equally important. Another point of significance is that the pathway analysis treats the van der Waals contact between Val31 and Trp48 as a single point-to-point interaction only, between $C_{\gamma 1}$ of valine and $C_{\delta 2}$ of tryptophan. However, from the refined structure coordinates of azurin [13], at least six of the atoms on the indole ring are in close contact (≤ 0.43 nm) with the valine side chain. A final point which makes the "Trp pathway" potentially interesting is the possible effect of a conjugated π-orbital system on the electronic coupling. The pathway calculation, however, only takes σ-bonds into account.

In order to examine the possible influence of aromatic residues on ET we have produced single-site-mutated azurins where Trp48 has been substituted by other amino acids, both aromatic and non-aromatic, and determined the rate constants for intramolecular ET and its activation parameters [9, 12]. The results are shown in Table 1 together with the standard free energies of reaction (ΔG^0), and demonstrate that substitution of Trp48 with other amino acids only has a negligible effect on the kinetic parameters when corrected for changes in driving force. However, an additional mutant was prepared where Val31 was substituted with Trp, thus producing a "double-Trp" azurin (V31W) [12] where the two indole rings are placed in neighbouring positions (cf. Fig. 1). Further, 2D NMR data demonstrate that the region in the mutant protein located between Trp 48 and the copper centre maintains the same structure as its equivalent in the WT protein. Energy minimization calculations on this mutant have also been performed and show a close (van der Waals) contact of the two indole rings consistent with observed NOEs between protons of the two ring systems [12]. LRET in the V31W azurin mutant was found to take place with a rate constant of $285\ s^{-1}$ at 298 K and pH 7.0, which is considerably faster than for any other azurin studied so far [6–12]. This strongly suggeststst that the dominant ET route is the "Trp48" pathway, since the alternative pathway through His46 would not be affected by this mutation. It should also be stressed that reoxidation of $RSSR^-$ and reduction of Cu(II) occur concomitantly without any resolvable intermediates, clearly excluding formation of amino acid radical intermediates and supports ET operation by electronic coupling.

Examination of the activation parameters for ET in V31W azurin provides some new insights: The increase in rate in V31W azurin is due to a more advantageous entropy of activation (Table 1), which is larger by 16.8 J $K^{-1}mol^{-1}$ than that of WT azurin. Since ΔS^0 is in all probability the same for intramolecular ET in WT and V31W azurin, the increase in activation entropy would according to Eq. 4 cause a *decrease* in $\beta(r–r_0)$ from the previously calculated value of 24.6 in WT to 22.6 in V31W azurin. A smaller electronic decay factor, β, in the mutant is also reflected in the electronic coupling energy, H_{DA}, between the electron donor and the electron acceptor, which we have calculated to be 2.1×10^{-7} eV. This is an improvement by a factor 2.6 relative to WT azurin ($H_{DA} = 0.8 \times 10^{-7}$ eV). In contrast, a calculation of the covalent tunnelling length gave $\sigma_l = 5.0$ nm for the "double Trp pathway" in V31W azurin as compared with $\sigma_l = 4.62$ nm for the "Trp48 pathway" in WT azurin (see above). Obviously, a different tunnelling length cannot explain the observation of the increased rate in this mutant.

We suggest that the relative positions of Trp31 and Trp48 may enhance the interaction between D and A since the ring systems are in van der Waals contact, which may provide a considerable electronic overlap and give rise to a resonance-type tunnelling through the indole rings. Aromatic residues placed in appropriate positions may enhance ET through proteins by a more effective coupling through their extended π^*-orbitals, since the energy gap between that of the tunnelling electron and the aromatic π-system is significantly smaller than that between the electron-tunnelling energy and σ-orbitals. Apparently, a single aromatic residue placed midway between D and A in a predominantly σ-ET pathway is not advantageous by itself, since $\sigma \rightarrow \pi \rightarrow \sigma$ ET will be energetically unfavourable. How-

ever, several aromatic residues placed in consecutive proximal positions or aromatic molecules in direct contact with either D or A may act as an extended relay which could enhance the electronic coupling.

LRET in blue copper oxidases

Evolutionary pressure is expected to lead to specific pathways in systems where intramolecular electron transfer is part of functional processes. Cytochrome *c* oxidase and ascorbate oxidase belong to a class of enzymes where sequential ET steps constitute part of the catalytic cycle (as distinct from the wide range of other redox enzymes catalysing atom transfer, e.g. the dehydrogenases).

High-resolution (0.19 nm) three-dimensional structures are now available for ascorbate oxidase (AO) in both oxidised and reduced states [34, 35]. AO, like all other blue oxidases, catalyses the reduction of O_2 to H_2O in four sequential single-electron transfer steps to the blue type-1 (T1) copper site, while dioxygen coordinates and becomes reduced at a trinuclear, type-2/type-3 (T2/T3) copper centre [36]. Obviously, intramolecular ET plays a central role in the physiological function of this enzyme. Under anaerobic conditions, the rate constant for intramolecular electron transfer from T1Cu(I) to T2/T3Cu(II) in AO was found to be only $200\ s^{-1}$ at room temperature [18], although the two redox centers are connected by a direct chemical bond pathway (cf. Fig. 2) with $\sigma_1 = 1.26$ nm between Cys507(S_γ) and His506/508(N). The reason for the observed slow ET process is at least twofold: (a) under anaerobic conditions the driving force is close to zero, and (b) the reorganisation energy was found to be considerable, 142 ± 10 kJ mol^{-1} [18]. Clearly, the physiological process of dioxygen reduction is gated by either substrate (O_2) binding and/or conformational changes at the trinuclear site. Indeed, both coordinationof O_2 to the trinuclear centre [19] and oxidising the fully reduced enzyme [37] were found to markedly enhance rates of intramolecular ET.

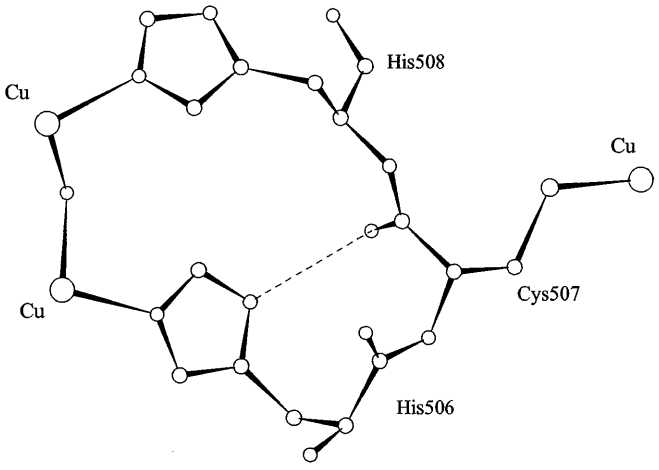

Fig. 2 Calculated pathways for ET from T1Cu(I) (*right* coordinated to Cys507) to the T3Cu(II) centre (*left* coordinated to His506/508) in ascorbate oxidase [18]. The coordinates were taken from the Brookhaven Protein Databank [34]

While the structural changes that were resolved between fully oxidised and reduced AO may exclude some of the internal ET steps from being non-adiabatic, this may not be the case in the initial ET step of cytochrome *c* oxidase reduction. For the latter case a rate constant of $2 \times 10^4\ s^{-1}$ has been observed [38], whereas the analogous step in the former oxidase is 100 times slower. The shortest distance separating Cu_A and heme-*a* in cytochrome *c* oxidase is 2.0 nm through a hydrogen bonding system including His504 and Arg438/439 [39], which is quite a noteworthy distance for a physiological ET system. In AO the T1-T3 distance between Cys507(S_γ) and His506/508(N) is only 1.3 nm, underscoring the marked difference in rates contrasted by the inverse separation distance.

Conclusions

While azurins turned out to provide a very advantageous system for examining the parameters which control LRET in a β-sheet polypeptide matrix that was not a product of evolutionary selection, ascorbate oxidase provides such an example. Both proteins are structurally well-characterized macromolecules, consisting of a very rigid β-sheet polypeptide matrix to which the redox centres are directly connected without intervening cofactors. Moreover, the fact that the electron donor and electron acceptor constitute an integral part of the protein provides the additional advantage of enabling a rigorous definition of separation distances. The increasing body of data for intramolecular LRET in blue copper proteins convincingly supports mechanisms where definined pathways are operative.

Acknowledgements This research has been supported by the Danish Natural Science Research Council, the VW Foundation and the German-Israeli Foundation (I 320-211-05). This support is gratefully acknowledged. We also wish to thank Dr. Lars K. Skov for producing the figures for this paper.

References

1. Beratan DN, Onuchic JN, Winkler JR, Gray HB (1992) Science 258:1740–1741
2. Moser CC, Keske JM, Warncke K, Farid RS, Dutton PL (1992) Nature 355:796–802
3. Farid RS, Moser CC, Dutton PL (1993) Curr Opin Struct Biol 3:225–233
4. Siddarth P, Marcus RA (1993) J Phys Chem 97:13078–13082
5. Gray HB, Winkler JR (1996) Ann Rev Biochem 65:537–561
6. Farver O, Pecht I (1989) Proc Natl Acad Sci USA 86:6968–6972
7. Farver O, Pecht I (1992) J Am Chem Soc 114:5764–5767

8. Farver O, Skov LK, Kamp M van de, Canters GW, Pecht I (1992) Eur J Biochem 210:399–403
9. Farver O, Skov LK, Pascher T, Karlsson BG, Nordling M, Lundberg LG, Vänngård T, Pecht I (1993) Biochemistry 32:7317–7322
10. Farver O, Skov LK, Gilardi G, Pouderoyen G van, Canters GW, Wherland S, Pecht I (1996) Chem Phys 204:271–277
11. Farver O, Bonander N, Skov LK, Pecht I (1996) Inorg Chim Acta 243:127–133
12. Farver O, Skov LK, Young S, Bonander N, Karlsson BG, Vänngård T, Pecht I (1997) J Am Chem Soc (in press)
13. Nar H, Messerschmidt A, Huber R, Kamp M van de, Canters GW (1991) J Mol Biol 221:765–772
14. Baker EN (1988) J Mol Biol 203:1071–1095
15. Nar H, Messerschmidt A, Huber R, Kamp M van de, Canters GW (1991) J Mol Biol 218:427–447
16. Romero A, Hoitink CWG, Nar H, Huber R, Messerschmidt A, Canters GW (1993) J Mol Biol 229:1007–1021
17. Hammann C, Messerschmidt A, Huber R, Nar H, Gilardi G, Canters, GW (1996) J Mol Biol 255:362–366
18. Farver O, Pecht I (1992) Proc Natl Acad Sci USA 89:8283–8287
19. Farver O, Wherland S, Pecht I (1994) J Biol Chem 269:22933–22936
20. Marcus RA, Sutin N (1985) Biochim Biophys Acta 811:265–322
21. Therien MJ, Chang J, Raphael AL, Bowler BE, Gray HB (1991) In: Clarke MJ et al. (eds) Long range electron transfer in biology (Structure and bounding, vol. 75). Springer, Berlin Heidelberg New York, pp 110–129
22. Winkler JR, Gray HB (1997) JBIC 2:399–404
23. Langen R, Colon JL, Casimiro DR, Karpisin TB, Winkler JR, Gray HB (1996) JBIC 1:221– 225
24. Regan JJ, Risser SM, Beratan DN, Onuchic JN (1993) J Phys Chem 97:13083–13088
25. Skourtis SS, Regan JJ, Onuchic JN (1994) J Phys Chem 98:3379–3388
26. Langen R, Chang I-J, Germanas JP, Richards JH, Winkler JR, Gray HB (1995) Science 268:1733–1735
27. Broo A, Larsson S (1990) Chem Phys 148:103–115
28. Jackman MP, Sykes AG, Salmon GA (1987) J Chem Soc Chem Commun 1987:65–66
29. Jackman MP, McGinnis J Powls R, Salmon GA, Sykes AG (1988) J Am Chem Soc 110:5880–5887
30. Fields BA, Bartsch HH, Bartunik HD, Cordes F, Guss JM, Freeman HC (1994) Acta Cryst D50:709–730
31. Christensen HEM, Conrad LS, Mikkelsen KV, Nielsen MK, Ulstrup, J (1990) Inorg Chem 29:2808–2816
32. Lowery MD, Guckert JA, Gebhard MS, Solomon EI (1993) J Am Chem Soc 115:3012–3013
33. Larsson S, Broo A, Sjölin L (1995) J Phys Chem 99:4860–4865
34. Messerschmidt A, Ladenstein R, Huber R, Bolognesi M, Avigliano L, Marchesini A, Petruzzelli R, Rossi A, Finazzi-Agró A (1992) J Mol Biol 224:179–205
35. Messerschmidt A, Luecke H, Huber R (1993) J Mol Biol 230:997–1014
36. Farver O, Pecht I (1997) In: Messerschmidt A (ed) Electron transfer reactions in multi-copper oxidases. World Scientific (in press)
37. Hazzard JT, Marchesini A, Curir P, Tollin G (1994) Biochim Biophys Acta 1208:166–170
38. Nilsson T (1992) Proc Natl Acad Sci USA 89:6497–6501
39. Tsukihara T, Aoyama H, Yamashita E, Tomizaki T, Yamaguchi H, Shinzawa-Itoh K, Nakashima R, Yaono R, Yoshikawa S (1996) Science 272:1136-1144