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Biochimica et Biophysica Acta 1655 (2004) 59–63
BIOCHIMICA ET BIOPHYSICA ACTA
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Review

Electron tunneling in rhenium-modified *Pseudomonas aeruginosa* azurins

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Received 24 April 2003; received in revised form 26 June 2003; accepted 26 June 2003

Abstract

Laser flash-quench methods have been used to generate tyrosine and tryptophan radicals in structurally characterized rhenium-modified *Pseudomonas aeruginosa* azurins. Cu(I) to "Re(II)" electron tunneling in Re(H107) azurin occurs in the microsecond range. This reaction is much faster than that studied previously for Cu(I) to Ru(III) tunneling in Ru(H107) azurin, suggesting that a multistep ("hopping") mechanism might be involved. Although a Y108 radical can be generated by flash-quenching a Re(H107)M(II) (M = Cu, Zn) protein, the evidence suggests that it is not an active intermediate in the enhanced Cu(I) oxidation. Rather, the likely explanation is rapid conversion of Re(II)(H107) to deprotonated Re(I)(H107 radical), followed by electron tunneling from Cu(I) to the hole in the imidazole ligand.
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Keywords: Electron tunneling; Rhenium complex; Amino acid radical; Blue copper; Azurin

At ICBIC-9 in Minneapolis, we reported that electron tunneling from Cu(I) to a "Re(II)" complex attached to His-107 in a structurally characterized Re-modified *Pseudomonas aeruginosa* azurin is several hundred times faster than the corresponding reaction to a similar Ru(III)(H107) center [1]. We suggested that this enhanced rate might be due to multistep tunneling ("hopping"), with a tyrosine radical (Y108) serving as an active intermediate in the Cu(I) oxidation, according to the scheme shown in Fig. 1 [2,3].

Jerry Babcock, who was in the audience, jumped up and asked if we had seen the tyrosine radical optically or by EPR. We admitted that we had not, but promised Jerry that we would work on experiments to see if we could.

Soon after ICBIC-9, urged on by Jerry, we began to study amino acid radicals in structurally characterized protein environments with emphasis on Re azurins (ReAz). Until his untimely death in December 2001, Jerry greatly assisted our efforts through advice and encouragement (he was our favorite cheerleader); with deep affection, we dedicate this progress report to his memory.

1. Preparation of Re azurins

Rhenium-modified *P. aeruginosa* azurin can be prepared by methods analogous to those we have employed for Ru proteins [3]. A single surface histidine on each azurin mutant binds to a rhenium(I)tricarbonyl(1,10-phenanthroline) [$Re(CO)_3$(phen)] unit by displacing water from the labeling reagent, [$Re(CO)_3$(phen)(H_2O)] (triflate). Each ReAz is readily purified by metal affinity chromatography and anion/cation exchange chromatography. The Re(I) and Cu(II) centers are not strongly coupled, as evidenced by the absorption spectrum of Re(H107)Az, which is a superposition of the blue copper spectrum and the Re(I) spectrum (Fig. 2).

2. Reduction potentials

Cyclic voltammetry of [$Re(CO)_3$(phen)(imidazole)]$^+$ (sulfate salt) in acetonitrile shows a single, quasi reversible

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doi:10.1016/j.bbabio.2003.06.010

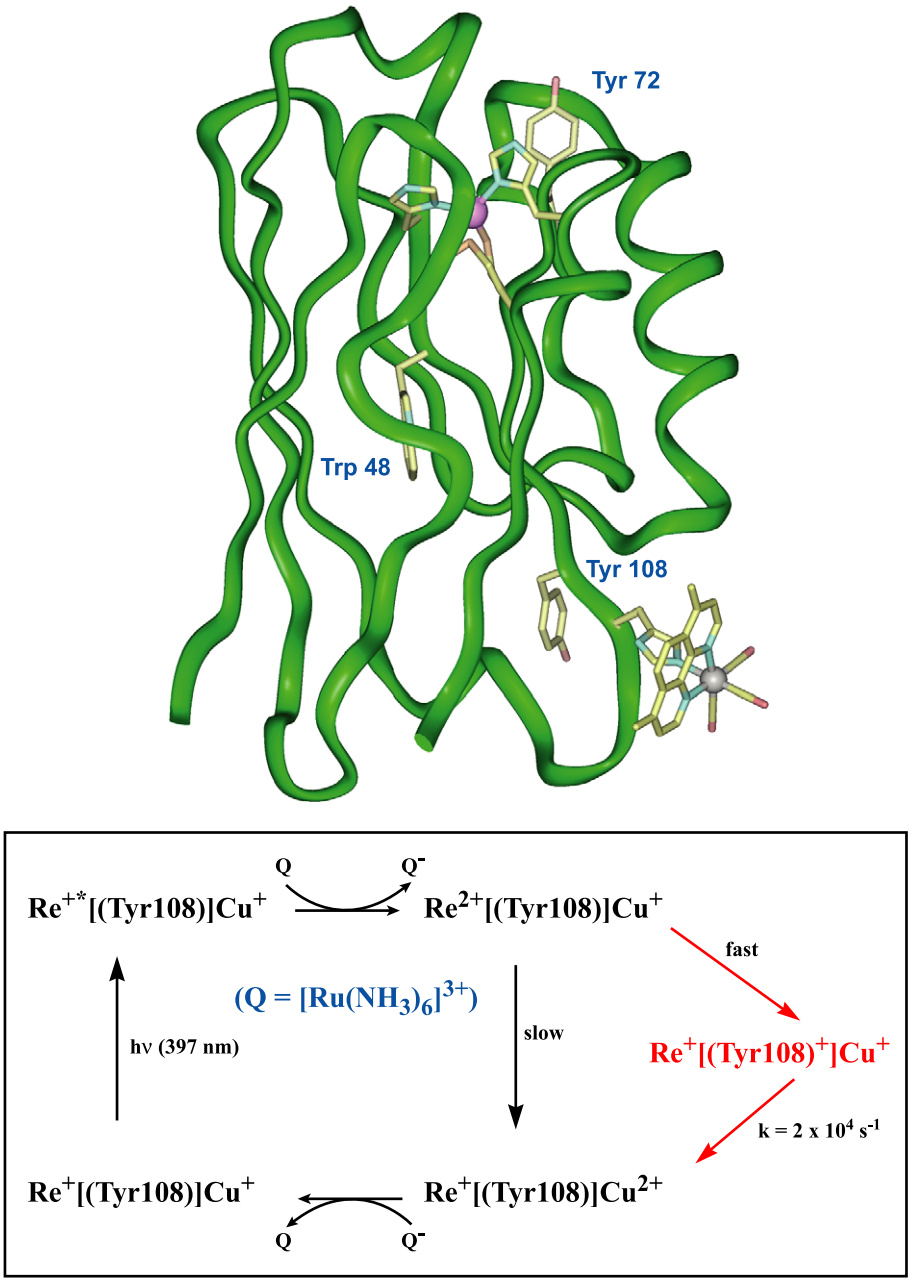

Fig. 1. Structure of Re-labeled H83Q/Q107H azurin [Re(H107)Az] and a proposed hopping mechanism for Cu(I) oxidation [2,3].

wave at 1.85 V vs. NHE [4]. The one-electron reduction of the complex occurs at −1.06 V vs. NHE in acetonitrile. The modified Latimer diagram for the Re(I) complex shows that it is a powerful excited-state oxidant [Re(I)*/Re(0) ~ 1.3 V] as well as an excited-state reductant [Re(II)/Re(I)* ~ −0.5 V] (Fig. 3).

3. The "Re(II)" oxidant

The excited state [Re(I)*] has a lifetime of 70 ns, allowing bimolecular ET to an exogenous electron acceptor to take place [2,5]. Irreversible reaction of Re(I)* with a Co(III) electron acceptor leads to formation of an oxidized rhenium fragment. DFT calculations of "Re(II)" indicate that the unpaired electron in this species has density on both the imidazole and the metal center. When the imidazole is protonated, most of the spin density of the radical resides on the rhenium (81%), but when the imidazole is deprotonated, as would be the case at pH 7, the spin density is largely on the imidazole (71%) (Fig. 4). Both of these "Re(II)" complexes are powerful oxidants; indeed, either should be able to generate a tyrosine or a tryptophan radical, whose potential is lower [6,7].

4. Cu(I) to "Re(II)" electron tunneling

The rate of electron tunneling through RuAz drops off exponentially with distance, with a decay constant $\beta \sim 1.1$ $Å^{-1}$ (green dots, Fig. 5A) [2,8–12]. Analogous rates for ReAz are higher for every tunneling mutant examined (red dots, Fig. 5A). Initially, this finding puzzled us, as ET reactions in azurin at very high driving forces are predicted to be in the inverted region ($-\Delta G^0 > \lambda$) [13–15]. Inverted behavior, however, can be avoided if hopping occurs, as in the proposed mechanism shown in Fig. 1.

To determine whether photogenerated "Re(II)" is capable of oxidizing an amino acid along an ET pathway, Zn(II)

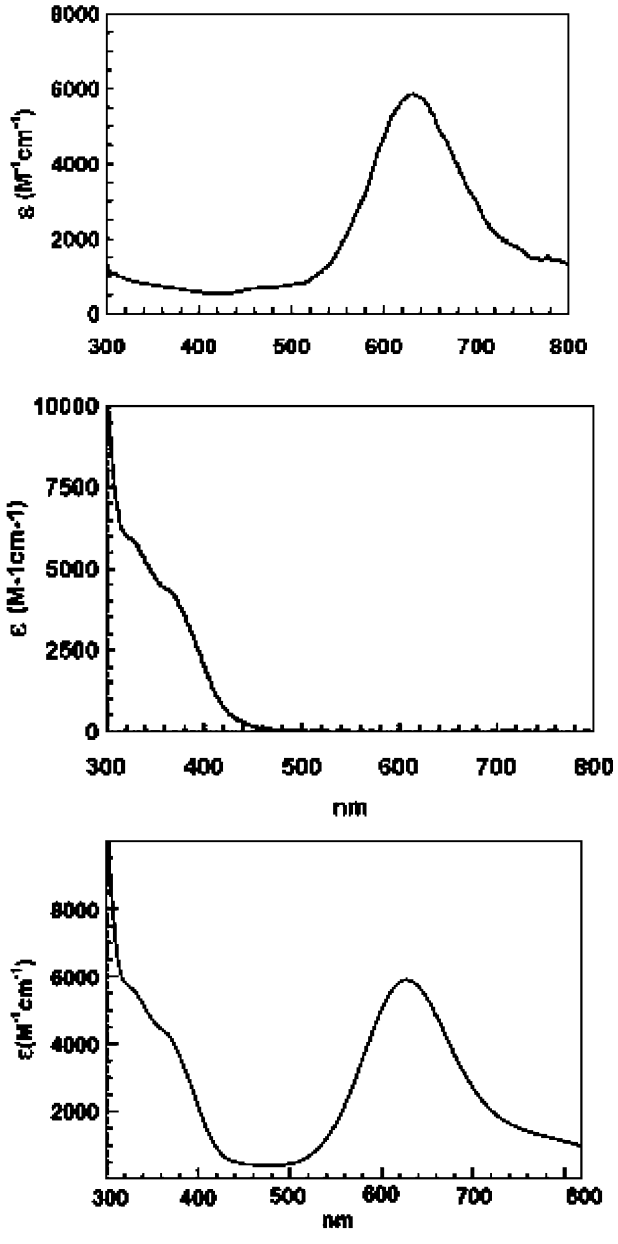

Fig. 2. Solution absorption spectra in 50 mM KPi buffer, pH 7.2: (A) unlabeled H83Q/Q107H-Az, (B) $[Re(CO)_3(phen)(imidazole)]^+$(sulfate salt), and (C) Re(H107)Az.

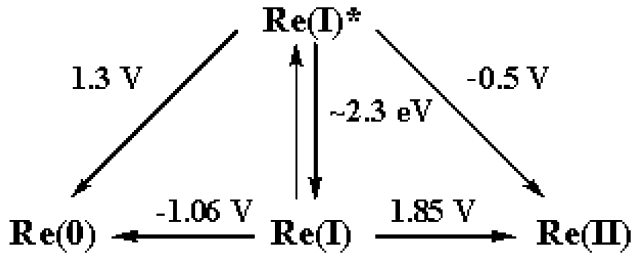

Fig. 3. Modified Latimer diagram for $[Re(CO)_3(phen)(imidazole)]^+$ (potentials vs. NHE). Data from Ref. [4].

derivatives of Re(H83)Az and Re(H107)Az were studied. After flash/freeze/quench, the EPR spectrum of Re(H83)Az exhibited a signal attributable to a W48 radical [3], and the spectrum of Re(H107)Az was clearly that of a tyrosine radical [3,16].

To test the idea that $Y108^{\bullet}$ is an active intermediate in the Cu(I) to "Re(II)(H107)" reaction, Cu(I) oxidation in Re-labeled H83Q/Q107H/W48F/Y72F/Y108F [Re(H107)(all Phe)Az] and H83Q/Q107H/W48F/Y72F/Y108F/F110W [Re(H107)(W110)Az] were examined by monitoring the appearance of blue Cu(II) absorption at 632 nm (Fig. 6). In both cases, the ET rate constants are roughly 10^4 s^{-1}, clearly showing that Y108 is not involved in rapid Cu(I) to "Re(II)" tunneling.

So what is responsible for the rapid Cu(I) to "Re(II)" reaction in Re(H107)(all Phe)Az (Fig. 6B)? An attractive possibility is tunneling directly to the coordinated histidine radical, as the imidazole hole (Fig. 4B) should be more strongly coupled to the backbone than the metal ion. Indeed, this explanation accounts nicely for the similar rates observed for all three Re(H107) azurins (Fig. 6). Thus, we propose in each case that the active oxidant is a photogenerated histidine radical, noting that Cu(I) oxidation rates replotted against distances measured from Cγ of all tunneling-mutant surface histidines (Fig. 5B) are in strikingly good agreement with those predicted based on the standard 1.1 $Å^{-1}$ protein tunneling decay constant.

We have much work left to do before we can say with certainty that a coordinated histidine radical is the active oxidant in the Cu(I) to "Re(II)" electron tunneling reaction. We have observed a signal at X-band that appears to be that of a histidine radical [17] after flash/freeze/quench experiments on Re(H107)(all Phe)Az, but we need to examine the EPR spectrum of this radical at high fields as well as by ENDOR and other spectroscopic methods before assigning it definitively. We are also planning time-resolved EPR experiments to establish whether or not the "His radical" is the acceptor in the Cu(I) to "Re(II)" tunneling reaction.

As a parting note, directed mainly to "Mom Babcock", we can say in the spirit of the 2002 MSU memorial symposium that we wish Jerry were here to collaborate with us in the next phase of our work on multistep tunneling reactions. We could use his help!

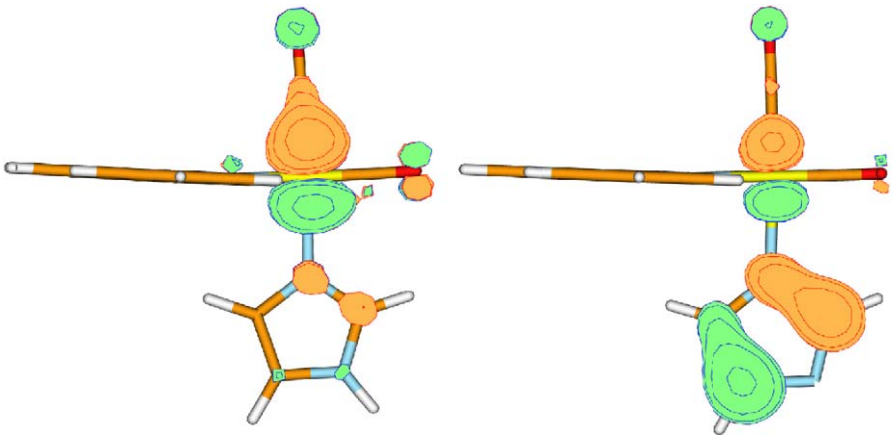

Fig. 4. Natural orbitals for the singly occupied levels of (A) $[Re(II)(CO)_3(phen)(imidazole)]^{2+}$ and (B) $[Re(II)(CO)_3(phen)(imidazolate)]^{+}$, showing radical character moving from the Re to the imidazole upon ligand deprotonation. Spin densities (A/B) are Re (0.81/0.25), imidazole (0.09/0.71). Structures were optimized at the B3LYP/LANL2DZ level using Gaussian 98 (Revision A.11.4).

Acknowledgements

Our research on electron tunneling in proteins is supported by NIH grant DK19038.

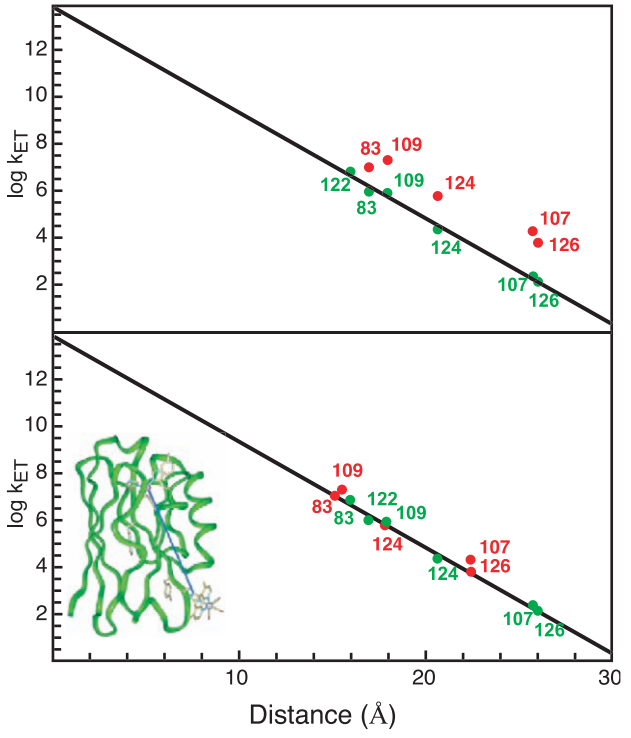

Fig. 5. (A) Electron tunneling timetables for Cu(I) to Ru(III) and "Re(II)" reactions in azurins: (A) Ru-Cu (●) and Re-Cu (●) distances; (B) Ru-Cu (●) and Cγ(histidine)-Cu [ReAz] (●) distances. Inset: Cγ-Cu distance in Re(H107)(Y108)Az. Numbering corresponds to surface histidine positions. Distances are from crystal structures or models derived from crystal structures [2,3,9,11,12,15].

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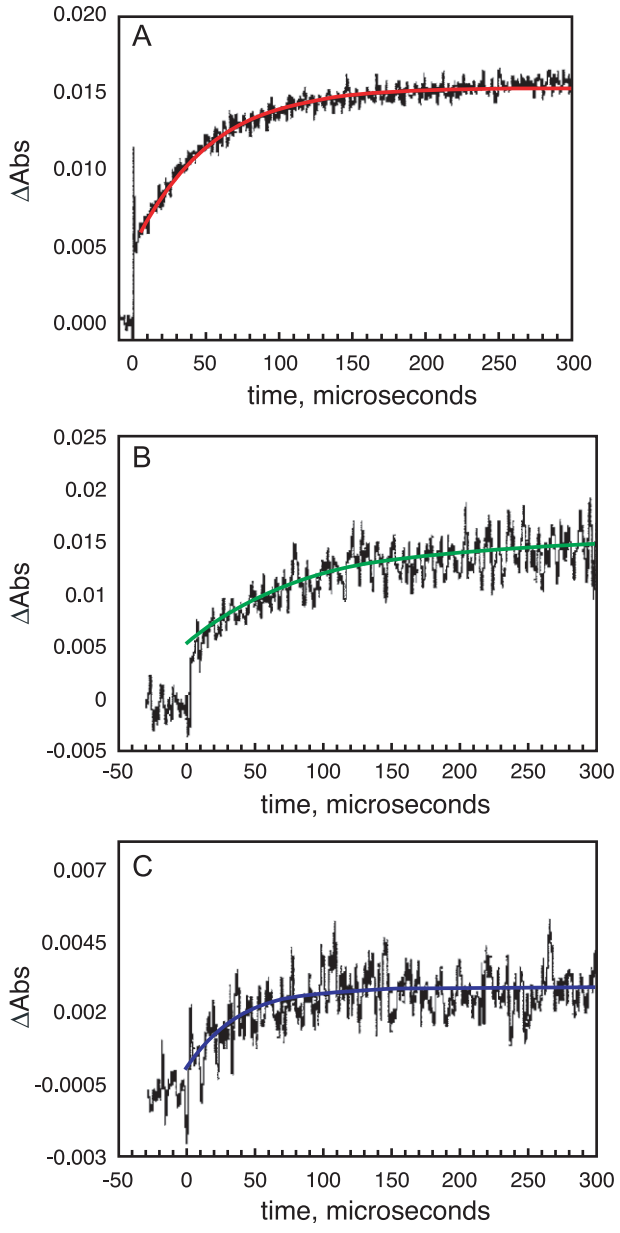

Fig. 6. Kinetics of Cu(I) oxidation by "Re(II)": (A) Re(H107)Az (● fit with $k=2\times10^4$ s^{-1}); (B) Re(H107)(all Phe)Az (● fit with $k=1\times10^4$ s^{-1}); and (C) Re(H107)(W110)Az (● fit with $k=3\times10^4$ s^{-1}, protein concentration range 26–47 μM). All fittings were by a linear least squares method.

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