Preparation of a Penta-ammineruthenium(III) Derivative of *Chromatium vinosum* HIPIP and the Kinetics of Intramolecular Electron Transfer

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The rate constant (~20 °C) for intramolecular electron transfer from $Ru(NH_3)_5^{2+}$ attached at the Histidine-42 of HIPIP to the [4Fe–4S] (oxidised) active site, distance 7.9 Å, is 18 s^{-1}.

Reaction of $[Ru(NH_3)_5H_2O]^{2+}$ with the high-potential iron–sulphur protein from *Chromatium vinosum* results in attachment of the Ru label to the Histidine-42 residue. Using pulse radiolysis techniques the rate constant for intramolecular electron transfer from Ru^{II} to the [4Fe–4S] core has been determined. Since His42 is attached to Cys43 (which is bound to the cube), a through-bond distance of <15 Å exists between the donor and acceptor sites. This is the first instance in such studies of a short direct linkage between the redox sites in which 'through-bond' as opposed to 'through-space' electron transfer is a possibility.

HIPIP from *C.vinosum* is a small protein (M_r 9257) containing a single cuboidal Fe_4S_4 cluster covalently bonded to four cysteinyl residues of a single polypeptide chain (85 amino acids).[1] The high reduction potential ($E°$ 350 mV *vs.* normal hydrogen electrode at pH 7.0)[2] contrasts with that of related ferredoxins ($E° \sim -400$ mV).[3] HIPIP was isolated and purified as described by Bartsch.[2] The reduced protein was characterised by its u.v.–visible peak at 388 nm, ε 16.0 × 10^3 $M^{-1}cm^{-1}$.[4]

The aquapenta-ammineruthenium(II) complex, $[Ru(NH_3)_5H_2O](PF_6)_2$ was prepared by a published procedure,[5] and the formula confirmed by analysis. Reaction of $[Ru(NH_3)_5H_2O]^{2+}$ with HIPIP and separation of products were carried out by a procedure similar to that already described for plastocyanin.[6] The major product (~80%) was further purified by anion exchange chromatography. The Ru : Fe ratio for this product determined by inductively coupled plasma (ICP) atomic emission spectroscopy, is 1 : 4 (±2%), consistent with a mono-ruthenated product. The sharp C_2H resonance of His42 in the ^1H n.m.r. spectrum (δ 8.3), is absent in the spectrum of the paramagnetic semi-reduced Ru^{III} modified form, Ru^{III}–$HIPIP_{red}$. Also, reaction of DEPC (diethylpyrocarbonate)[7] with the His42 of native HIPIP (peak at 240 nm, Δε ~3200 M^{-1} cm^{-1}) is no longer observed for the Ru-modified protein. It is concluded therefore that $Ru(NH_3)_5$ attachment is exclusively at the His42 site.

It has been demonstrated that the Ru-modified protein has an $E°$ of 350 ± 10 mV for the $[4Fe–4S]^{3+/2+}$ change (same as for the native protein). Also the u.v.-visible spectra of both redox states (350—700 nm) are indistinguishable from those for native HIPIP. Furthermore, the hyperfine contact shifted n.m.r. resonances of protons adjacent to cluster ligands remain virtually unperturbed by Ru modification.

Pulse radiolysis studies were carried out on oxidised ruthenated HIPIP in 0.10 M phosphate buffer (pH 7.0) prepared in O_2-free triply-distilled water under argon. Tertiary butanol (0.4 M) was added to scavenge H and OH radicals so that e^-_{aq} was the sole reducing species. The dose was such that <20% of protein was reduced in any one pulse. Progress of the reaction was monitored at 480 nm. The [4Fe–4S] chromophore, but not the Ru, absorbs at this wavelength. Direct reaction of e^-_{aq} with HIPIP is observed as an initial step on the ms time scale.[8] This rapid stage corresponds to the direct reduction of the Ru^{III} or Fe_4S_4 centre as in reactions (1) and (2). A second reaction stage is observed with Ru-modified, but not native, HIPIP. In this, the first order decay is independent of protein concentration (varied 2-fold by dilution and 5-fold by successive pulsing), and is assigned to an intramolecular process (3). The rate constant is 18 ± 2 s^{-1}. A third stage (s time scale) is observed for Ru-modified HIPIP,

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which is also absent with native protein, and is assigned to the bimolecular process (4). This stage stems from the build-up of the autoreduced species Ru^{III}–$HIPIP_{red}$, which reacts with e^-_{aq} to give Ru^{II}–$HIPIP_{red}$. On addition of native $HIPIP_{ox}$ a more rapid third stage is observed consistent with this interpretation.

$$Ru^{III}\text{–}HIPIP_{ox} \rightarrow Ru^{II}\text{–}HIPIP_{ox} \quad (1)$$

$$Ru^{III}\text{–}HIPIP_{ox} \rightarrow Ru^{III}\text{–}HIPIP_{red} \quad (2)$$

$$Ru^{II}\text{–}HIPIP_{ox} \longrightarrow Ru^{III}\text{–}HIPIP_{red} \quad (3)$$

$$Ru^{II}\text{–}HIPIP_{red} + Ru^{III}\text{–}HIPIP_{ox} \rightarrow 2Ru^{III}\text{–}HIPIP_{red} \quad (4)$$

For the intramolecular electron-transfer process (3), the edge to edge through-space distance (d) between the two redox centres is estimated using molecular graphics to be 7.9 Å, which is the distance from the $^{\delta}C$ of His42 to the nearest μ_3-S of the [4Fe–4S] cube. The direct although more circuitous through-bond distance to the cysteinyl S atom is estimated to be around 13 Å.

The thermodynamic driving force (ΔE° ~270 mV) for (3) is similar to that for the corresponding intramolecular processes in Ru-modified cytochrome c,[9,10] azurin,[11] and plastocyanin,[12] all of which coincidentally have d ~12 Å. If a relationship of the kind $\ln k$ *vs.* d applies (with the same β and k_0),[13,14] then for d = 8.3 Å in the HIPIP case, k should be substantially (~10^2 times) bigger than 18 s^{-1}. There is therefore at this time no evidence for an enhancement in k favouring through-bond, as opposed to through-space, electron transfer. For through-space electron transfer the exceptionally small intramolecular rate constant observed for Ru-modified *Anabaena variabilis* and *Scenedesmus obliquus* plastocyanins[12] suggests that intervening polypeptide material also has a major controlling influence.

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