Long-Range Electron Transfer in Cobalt-Labeled Cytochrome *c*

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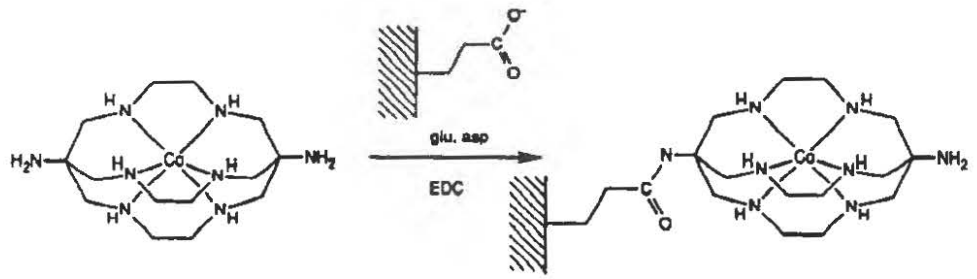
Long-range electron transfer reactions are crucial steps in many fundamental life processes. Cell respiration, for example, relies on the stepwise oxidation of organic substrates via a cascade of redox reactions. In photosynthetic organisms, chains of electron carriers such as flavoproteins, iron-sulfur proteins, and cytochromes participate in light-induced electron transport. Many of these oxidation/reduction reactions involve the transfer of an electron between redox sites which are separated by more than 10 Å of intervening protein matrix. Elucidation of the factors which control the rates of such long-range electron transfers has become the focus of extensive physical and biological investigation [1-7].

One factor that is known to be important is the distance separating the redox centers of the protein and the substrate in the activated complex undergoing electron transfer [4,5,8-10]. The first goal of this research project was to prepare and characterize a donor/acceptor system which would allow this distance dependence to be studied in a systematic fashion. The approach that we have taken involves the covalent attachment of a redox-active inorganic complex to specific surface residues on crystallographically characterized metalloprotein. This generates a semi-synthetic electron transfer system in which the distance and intervening medium between redox sites are fixed and known. By labelling the protein at multiple surface sites with the same metal complex, several donor/acceptor distances can be studied in a single protein. The idea is to measure and compare the intramolecular electron transfer rates for a series of modified proteins, in which the donor and acceptor, and the reaction free energy for electron transfer, remain constant.

Horse heart cytochrome c provided a convenient molecular framework upon which to base our work. This readily available, water-soluble component of the mitochondrial respiratory chain already contains one structurally well-characterized redox site in the form of a covalently attached heme c prosthetic group. A second redox site was introduced onto the protein surface by covalent attachment of the macrocyclic cage complex, $[Co(diAMsar)]^{3+}$, first prepared by Sargeson *et al.* [11], to several acidic residues. The attachment procedure utilized was the familiar carbodiimide-assisted coupling reaction, first applied to protein systems by Hoare and Koshland as a method of carboxylate quantitation [12]. In this reaction 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide (EDC) was used to promote the condensation of carboxylates provided by the protein with pendant amine groups furnished by the cobalt complex, resulting in a stable amide-bond linkage (Scheme 1).



Scheme 1. EDC-promoted coupling of Co(diAMsar) to surface carboxylates on cytochrome *c*.

The resulting isomeric singly-modified proteins were purified to homogeneity and characterized, in order to establish the unique modification site in each of the derivatives. Molecular modelling studies have shown that the resulting heme edge-to-cobalt distances span a range of 10 to 20 Å. The results of flash photolysis experiments designed to measure the intramolecular electron transfer rate constants (k_{et}) from Co(II) → Fe(III) in the thermodynamically-unstable mixed-valence state have been determined [13]. Surprisingly, the rates are essentially constant and are independent of the distance.

The modified proteins were also covalently attached to the surface of an edge-plane pyrolytic graphite electrode in an effort to measure k_{et} electrochemically using a second independent technique [14]. Although this approach was unsuccessful in yielding a rate constant, due to the presence of background currents which obscured the Faradaic process of interest, new directional attachment methodologies were developed. The ability to immobilize a protein in a specific molecular orientation has potential applications in biosensors [15]. In addition, spacially organized assemblies of redox proteins serve as models of biological electron transfer chains.

References

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