Electron Transfer Reactions of Cytochrome c

Jim Schwartz Final Seminar October 9, 1986

Cytochrome c is a small protein (104 amino acid residues, MW 12,400), some variant of which is found in almost all living organisms. The single polypeptide chain wraps itself around a heme prosthetic group forming a relatively spherical protein with the heme almost completely encapsulated. One edge of the heme is partially accessible to solvent water molecules and is named the "exposed" heme edge, the part of the protein incorporating this edge is called the heme crevice.

Cytochrome c functions as a mobile electron carrier, shuttling electrons between the two large, membrane-bound proteins cytochrome c reductase and cytochrome c oxidase. The iron of the heme of cytochrome c cycles between Fe(II) and Fe(III) as the protein is reduced and oxidized, respectively. Despite crystal structures for cytochrome c in both the oxidized (1) and reduced (2) forms, no definitive mechanism for how this protein transfers electrons has been accepted. A mechanism would delineate the surface of cytochrome c responsible for binding reaction partners and an electron transfer pathway within cytochrome c tracing the route electrons travel to and from the heme iron.

An important feature of cytochrome c reactivity is its strongly cationic nature (3), dictated by the large amount of positively charged lysine amino acids, a few of which encircle the exposed heme edge. By making use of chemically modified derivatives of cytochrome c in which any one of a number of these lysine residues has been singly altered to neutralize its positive charge (4), the electrostatic properties of cytochrome c have been exploited to map an electron transfer locus.

Two series of inorganic complexes were used to test the reactivity of these modified cytochromes c relative to that of the native protein. Each series contained complexes varying in charge but maintaining structural similarity; one set functioned as oxidants of cytochrome c [cobalt(III) bipyridine-like complexes] while the other was of reductants (ferrous polyaminocarboxylate-type complexes).

Measurement of the electron transfer rates (by stopped-flow spectrophotometry) as a function of ionic strength for reactions between charged inorganic complexes and the modified proteins demonstrated the importance of electrostatics in cytochrome c reactivity. In general, decreasing the positive charge of cytochrome c (by modification) had the effect of decreasing the rate of reaction with negatively charged complexes; however, these same derivatives reacted faster with positively charged complexes. The magnitude of the rate alteration was dependent upon both the location of the altered protein charge and the specific complex redox partner.

Reaction of these same derivatives with neutral inorganic complexes identified some non-electrostatic effects of the neutralization of protein lysine residues. Since neutral complexes are unaffected by the electrostatic field of the protein, they were able to identify the altered reduction potentials of the cytochrome derivatives as well as some alteration of the hydrophobicity of the protein surface due to the modification.

A more accurate picture of the importance of specific lysine groups was obtained after correcting rate constants for the non-electrostatic factors mentioned above. The lysine groups surrounding the heme edge seem to play an important role in active site recognition, i.e., they serve to orient incoming molecules toward productice collisions.

References

1. Takano, T.; Dickerson, R. E. J. Mol. Biol. 1981, 153, 79-94.

2. Takano, T.; Dickerson, R. E. J. Mol. Biol. 1981, 153, 95-115.

3. Moore, G. R.; Eley, C. G. S.; Williams, G. Adv. Inorg. Bioinorg. Mech. 1984, 3, 1-96.

4. Smith, H. T.; Staudenmayer, N.; Millett, F. Biochemistry 1977, 16, 4971-4974.