Covalent Attachment of Coordinatively-saturated Metal Complexes to Horse Cytochrome c Lysines

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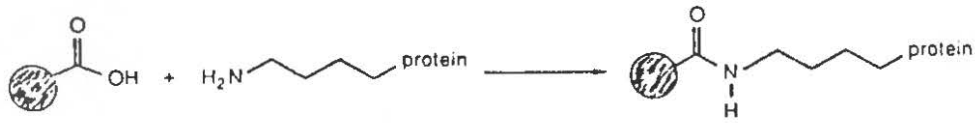
To date, most efforts to prepare proteins modified with inorganic metal sites have focused on systems in which a protein functionality is directly coordinated to the metal [1]. Because of the limited number of potential ligand functionalities present in the naturally occurring amino acids, such an approach limits the range of chemical and physical properties that can be designed into the modification site. The development of methods to attach intact metal complexes to a protein by reaction of a ligand-based functionality is one approach to removing some of these restrictions.

We have developed a method for covalently attaching the coordinatively saturated complexes $[Ru^{II}(NH_3)_5INH]^{2+}$ (INH = isonicotinic acid) and $Rubp_2$-cmbpH$]^{2+}$ (bp = 2,2'-bipyridine, cmbp = 4'-methyl-2,2'-bipyridine-4-carboxylic acid) to horse cytochrome c [2] via a carbodiimide-assisted condensation [3] of the pendant carboxylate of the complexes with the ε-amine of lysine to form an amide-bond linkage [4]:



The reaction of the activated complex $[Ru^{II}(NH_3)_5IN/EDC]^{3+}$, formed by the reaction of $Ru^{II}(NH_3)_5IN^{+}$ (pK_a = 2.75 + 0.10) with EDC (1-ethyl-3-(3-dimethylaminoproply)carbodiimide) at pH ~ 2.7, with cytochrome c at 6.0 < pH < 7.0 in ~ 1 M sodium phosphate gives a mixture of singly-modified proteins, $Ru(NH_3)_5IN$/cyt c, in ~ 10% yield. The $Ru(NH_3)_5IN$/cyt c products are separated by cation exchange chromatography. The total product yield and the product distribution is dependent on the reaction pH.

The $Ru(NH_3)_5IN$/cyt c products have been characterized by spectroscopic (optical absorption, difference spectroscopy, circular dichroism) and electrochemical methods (differential pulse voltammetry, spectroelectrochemistry). The physical properties of $Ru(NH_3)_5IN$/cyt c are the sum of the individual properties of native cytochrome c and the model complex $Ru(NH_3)_5ina^{2+/3+}$ (ina = isonicotinamide). Importantly, the native cytochrome c conformation is retained in the modified proteins. Tryptic peptide mapping experiments showed that the major reaction products are Lys-60-$Ru(NH_3)_5IN$/cyt c and Lys-22-$Ru(NH_3)_5IN$/cyt c. Models of the Lys-60 and Lys-22 proteins were constructed by molecular graphics. Based on the modeling studies, the enhanced reactivity of Lys-60 and Lys-22 towards $[Ru(NH_3)_5IN/EDC]^{3+}$ is postulated to arise primarily from electrostatic interactions.

Singly-modified $Rubp_2$cmbp/cyt c was prepared from $[Rubp_2cmbp/EC]^{3+}$ and cytochrome c by the same procedure used for $Ru(NH_3)_5IN$/cyt c. Preliminary peptide mapping results identify the site of modification of some of the components in the mixture of $Rubp_2$cmbp/cyts c. It appears that the product distribution is governed more by hydrophobic interactions between $[Rubp_2cmbp/EDC]^{3+}$ and cytochrome c than by simple electrostatic considerations.

References

1. For example:
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(b) Margalit, R.; Kostic, N. M.; Che, C.-m.; Blair, D. F.; Chiang, H. -J.; Pecht, I.; Shelton, J. B.; Shelton, J. R.; Schroeder, W. A.; Gray, H. B. Proc. Natl. Acad. Sci. U.S.A. 1984, 81, 6554.
(c) Peerey, L. M.; Kostic, N. M. Inorg. Chem. 1987, 26, 2079.

2. Four excellent reviews:
(a) Timkovich, R. In "The Porphyrins", vol. 7; Dolphin, D., Ed.; Academic: New York, 1979, p. 241.
(b) Ferguson-Miller, S.; Brautigan, D. L.; Margoliash, E. In "The Porphyrins", vol. 7; Dolphin, D., Ed.; Academic: New York, 1979, p. 149.
(c) Moore, G. R.; Eley, C. G. S.; Williams, G. Adv. Inorg. Bioinorg. Mech. 1984, 3, 1.
(d) Salemme, F. R. Ann. Rev. Biochem. 1977, 46, 299.

3. (a) Hoare, D. G.; Koshland, D. E. J. Am. Chem. Soc. 1966, 88, 2057.
(b) Hoare, D. G.; Koshland, D. E. J. Biol. Chem. 1967, 242, 2447.
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4. Very recently, Degani and Heller reported the modification of intersubunit lysine residues of glucose oxidase with ferrocenecarboxylic acid by an analogous condensation, the product containing 12 ferrocene units per protein:
(a) Degani, Y.; Heller, A. J. Phys. Chem. 1987, 91, 1285.