STUDIES ON CHEMICALLY MODIFIED CYTOCHROMES *c*

by

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ABSTRACT

Tuna and Horse cytochromes c were purified and chemically modified with the water soluble carboxyl group modifying reagent 1-ethyl-3-(3-dimethylamino-propyl) carbodiimide (EDC), using the method of Timkovich (1980). The time-course of modification was followed by visible spectroscopy and by functional measurements. Both methods indicated that disruption of the haem crevice regions of the proteins was largely complete within 10-15 minutes exposure to EDC.

The EDC modified Tuna and Horse proteins (THc* and HHc* respectively) showed essentially identical functional properties to those described by Timkovich (1980) for THc*. These include a typical methionine coordination to the haem iron, as shown by the absence of the 697nm visible absorption band from the spectra of the oxidized derivatives, and a pH dependent high spin - low spin transition for the derivatives in this oxidation state. In the reduced forms, THc* and HHc* show reactivity with carbon monoxide and oxygen indicating the haem crevice regions of these proteins is disrupted compared to those of the native proteins.

The tryptic peptides of native Tuna and Horse cytochromes c were mapped by HPLC and identified by amino acid analysis. Examination of the tryptic digests of THc* and HHc* by gel filtration and HPLC revealed the presence of trypsin indigestible material indicating the presence of EDC promoted intramolecular cross-links within the modified proteins. Failure to extract the haem groups from THc* and HHc* after cleavage of the protein-haem thio-ether bonds, indicates cross-linking of the exposed haem propionate groups to the proteins. Labelling of the native and EDC modified proteins with glycine methyl ester showed that THc* and HHc* contain several covalently modified carboxyl groups. The results of Timkovich (1980) for THc* are discussed with respect to these findings.

The reduction of THc*$^{3+}$ and HHc*$^{3+}$ by L-ascorbic acid and by the strong inorganic reducing agent sodium dithionite, was studied using stopped flow spectrophotometry. The kinetics of reduction of the modified proteins by these reagents could be accounted for by mechanisms similar to those proposed for the reduction of the native proteins by these reagents (Myer et al, 1980; Lambeth and Palmer, 1973). The effect on the reactions, of chemical modification of the proteins, is discussed.

The reaction of the reduced derivatives with carbon monoxide was studied by stopped flow spectrophotometry, flash photolysis, and by equilibrium binding measurements. The reduced derivatives have a high affinity for carbon monoxide. Flash photolytic studies indicated very low quantum yields for photodissociation of the reduced protein CO complexes.

The reduced derivatives formed complexes with oxygen which were unstable, decaying to form the oxidized protein. The rate of conversion to the oxidized protein was dependent on the solution pH. It was found that in the presence of excess reducing agent, THc^{*2+} and HHc^{*2+} could catalytically reduce oxygen. Steady state kinetic measurements were carried out to determine the dependence of the rate of oxygen reduction on various parameters. Similarities to the reactions of reduced carboxymethylated cytochrome c with oxygen are discussed.

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