EFFECTS OF P-CHLOROMERCURIPHENYL SULFONATE ON THE NONHEME IRON-PROTEIN OF THE REDUCED COENZYME Q - CYTOCHROME c REDUCTASE COMPLEX OF THE ELECTRON TRANSFER CHAIN OF BEEF HEART MITOCHONDRIA*

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In a previous report from this laboratory (Rieske, MacLennan and Coleman, 1963) the preparation and some of the properties of a nonheme iron-protein (in succinylated form) isolated from Complex III (reduced - Coenzyme Q - cytochrome c reductase) of the electron transfer chain of beef heart mitochondria were described. This protein is reddish-brown in color; upon reduction with ascorbate or dithionite a partial loss of absorption is observed. The addition of p-chloromercuriphenyl sulfonate (pcms) to the protein induces a loss of color greater than that due to either of these reducing agents and a complete loss of the characteristic absorption maxima on the long wavelength side of the protein peak. This report deals with some features of this phenomenon.

The absorption spectrum of the protein is shown in Fig. 1, before and after treatment with pcms. This figure also shows the absorption spectra of the protein after treatment with dithionite and with ascorbate. The loss of absorption induced by pcms is very pronounced. The small amount of absorption remaining is probably due in part to a small

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amount of an autoxidizable cytochrome with absorption maxima at 406 mμ in the oxidized form and at 416 mμ in the reduced form. The amount of this cytochrome varies from preparation to preparation.

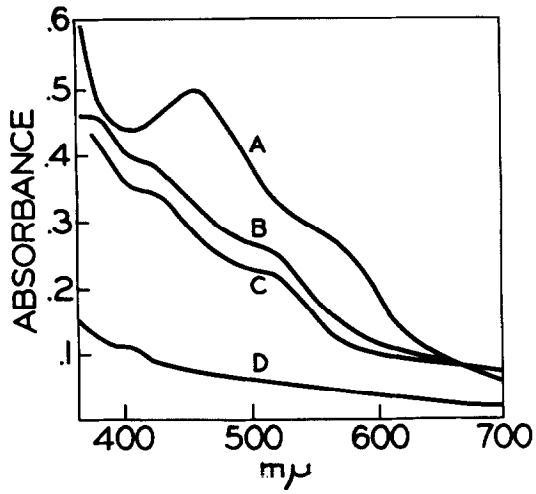

Fig. 1. Effect of pcms on the spectrum. A) Succinylated iron-protein (oxidized form 3.1 mg/ml in 0.1 M phosphate pH 7.5, 48 mμmole Fe/mg), B) as A but after addition of solid potassium ascorbate, C) as A but after addition of solid sodium dithionite, D) 1 ml of solution as in A + 0.05 ml of 0.01 M pcms (in 0.1 M phosphate pH 7.5) incubated for 30 min at 37°.

The extent of bleaching increases with increasing amounts of pcms until a point is reached beyond which additional pcms has no further effect. The time required for each addition of pcms to produce its maximal effect is dependent upon temperature; at 37° the reaction appears to be complete after 20 to 25 min; at lower temperatures a longer time is required. Mercaptide bond formation, as evidenced by an increase in absorption at 250 mμ (see Boyer, 1954), appears to accompany the bleaching phenomenon, the maximal effect being achieved by pcms in an amount closely similar to that required for maximal bleaching in the visible region. In the experiment shown in Fig. 2 (a and b), maximal effect was reached when the pcms added was equivalent to 2.2 moles per mole of protein-bound iron. Determinations of the equivalence of pcms to iron in other preparations have led to values of 1.64 and 2.1 for this ratio. The value

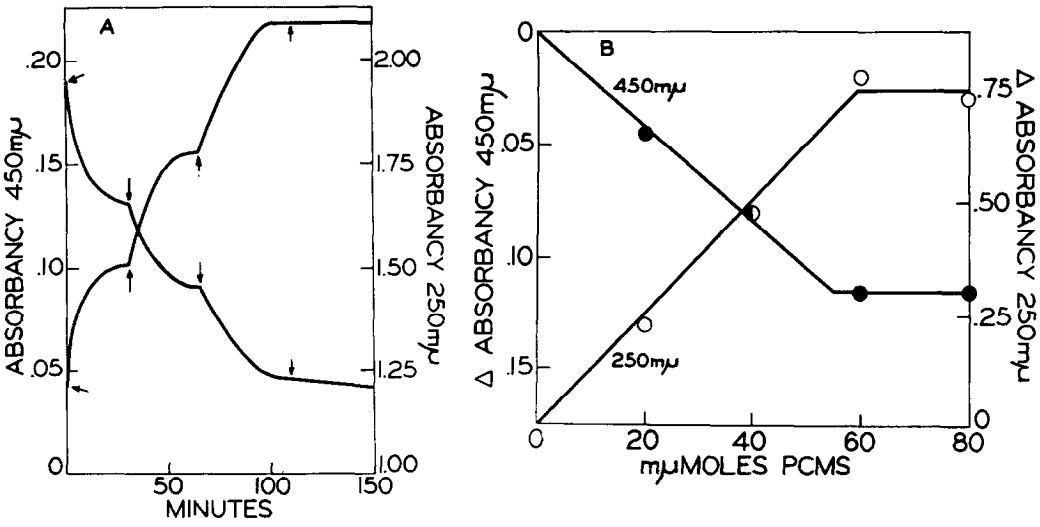

Fig. 2. a) Effect of pcms on visible (450 mμ) and ultraviolet (250 mμ) absorbance. Succinylated iron-protein (reduced form* 1.49 mg/ml in 0.2 M phosphate pH 7.5, 59.5 mμmole Fe/mg protein) 0.30 ml incubated at 37° with 0.002 ml 0.01 M pcms (in 0.2 M phosphate pH 7.5) added at intervals shown.

b) Data derived from this experiment after correction for contribution of added pcms to absorbance at 250 mμ and spontaneous bleaching at 450 mμ in absence of pcms.

*) Due to the spontaneous reduction of the fully oxidized protein the data reported in this communication refer mainly to the protein in a state of complete or almost complete reduction. Where studies have been possible with more highly oxidized material, the basic observations have been confirmed.

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of 1.64 is a minimal figure, however, since other experiments indicated that some degradation of the iron-protein had occurred in that particular sample.

The iron-protein was also decolorized by addition of Cu^{++} or Hg^{++} in an amount approximately stoichiometric with the iron. The iron-protein preparation used for these experiments required a pcms/Fe ratio of 1.64 for maximal bleaching; the analogous values obtained for Cu^{++} and Hg^{++} were 0.78 and 0.83 moles per mole iron, respectively. Katoh and Takamiya (1963), in a study of the iron-containing protein, photosynthetic pyridine nucleotide reductase from spinach, have indicated that heavy metal ions produced a bleaching effect similar to that of

p-chloromercuribenzoate and with a value of the ratio metal/iron required for maximal effect close to that reported in this communication.

Addition of the iron chelating reagent, Tiron, (disodium 1, 2, dihydroxybenzene - 3, 5 - disulfonate) after the protein had reacted with pcms, resulted in the production of the red color characteristic of the complex with ferric iron, with an absorption maximum at 490 mμ. The amount of color produced increased with increasing amounts of pcms preincubated with the protein up to a point slightly in excess of 2.2 moles pcms per mole of protein-bound iron (Fig. 3). The amount of color produced did not correspond with that calculated from the iron content of the preparation, assuming complete reaction. Color yield will depend, however, upon the valency state of the iron both before, and after, labilization. When Tiron was added after total bleaching by pcms, the red Tiron complex was found to separate from a colorless, almost iron-

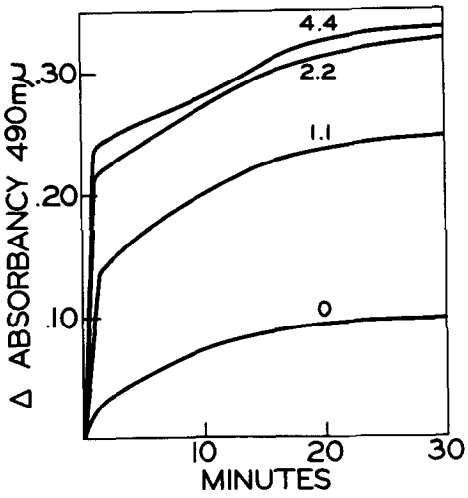

Fig. 3. Production of Tiron color after pcms. Succinylated iron-protein (partially oxidized form, 1.59 mg/ml in 0.1 M phosphate pH 7.5, 59.5 mμmole Fe/mg protein). 0.40 ml of this protein were reacted with 0.04 ml 0.1 M phosphate pH 7.5 containing appropriate amount of pcms calculated from Fe content of protein. Figures indicate molar ratios of pcms/Fe. After reaction for 20 min at 37°, Tiron, 0.04 ml of 0.1 M (in 0.1 M phosphate pH 7.5) was added.

Addition of pcms to the iron-protein caused a loss of the characteristic electron paramagnetic resonance (EPR) signal of this protein at g = 1.90. This signal has been tentatively associated with the presence of iron in the molecule (Beinert et al., 1962). Fig. 4 illustrates the results of an experiment in which the protein was incubated with pcms in amounts causing maximal and half-maximal bleaching before the EPR signal was measured. An amount of Cu^{++} giving maximal bleaching also produced the same effect as the higher amount of pcms, i.e. a loss of the signal.

The molecular arrangement which gives rise to the EPR signal at g = 1.90 may be a complex one. The present results indicate a correlation

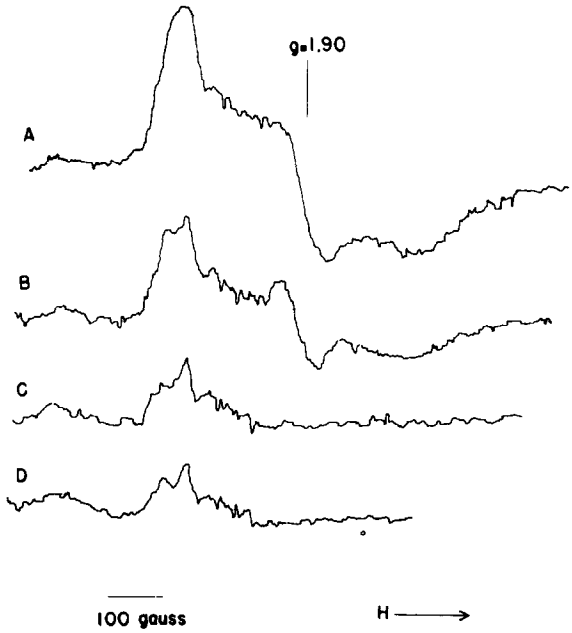

Fig. 4. Effect of pcms and heavy metal on EPR signal. Succinylated iron-protein, 2.50 mg/ml, (partially oxidized form in 0.1 M phosphate pH 7.5, 64 mμmoles Fe/mg protein). Maximal bleaching at 450 mμ for this sample occurred at pcms/Fe ratio of 2.1 moles pcms per mole Fe. A) Succinylated iron-protein alone. B) As A but with 1.06 mole pcms per mole Fe added before incubation. C) As A but with 2.1 mole pcms per mole Fe added. D) As A but with 1.06 mole Cu^{++} per mole Fe. All samples were incubated 37° 30 min then reduced with solid sodium dithionite before measuring the EPR signal. The signal shown is the first derivative (see Beinert, 1962). Temperature, -176°, Modulation amplitude 18 gauss, 25 mwatt microwave power.

between the labilization of iron produced by pcms and the loss of signal. The formation of mercaptide bonds during these changes may also indicate that sulphur compounds also have some role, either in determining the conformation of the molecule or in the binding of the iron to the protein. free protein during chromatography on Sephadex G-50; indicating that the iron is detached from the protein by these treatments. Treatment of the iron-protein with Cu^{++}, followed by addition of Tiron, also caused a production of the red complex.

If it becomes possible to isolate nonheme iron-proteins with characteristic EPR signals from the succinic and DPNH dehydrogenase regions of the electron transfer chain, then the behavior of these during incubation with pcms may give some indication of similarity or difference of molecular arrangement in this new class of proteins.

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