Crystalline Cytochrome c from Beef Heart Muscle

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For the past two years we have been concerned with the crystallization of beef cytochrome c. The yield of crystals by the alcohol precipitation method [1] is, however, very poor. In order to get a starting material for crystallization with reproducible properties we worked out the following preparation method *. 6 kg fresh cow hearts were minced, 15 liters of distilled water added and the pH adjusted to 4.1 with 1 N H_2SO_4. After passing cheese cloth the mince was extracted further with 6 liters of water, all at 4° C. The extract was neutralized with 4 N ammonia and centrifuged. 140 g of Amberlite XE-64 (in the ammonia form) were added to the supernatant. The resin with the adsorbed cytochrome was collected on a Büchner funnel, washed with 30 liters of distilled water and eluted with 2 500 ml 0.15 M phosphate of pH 7.0. The eluate was dialyzed against 0.002 M ammonia and centrifuged. The cytochrome was adsorbed again on 10 g of the same resin and eluted with a volume of 250–300 ml 0.09 M phosphate of pH 7.5. The eluate was saturated to 85 % with ammonium sulphate, centrifuged and filtered. The filtrate was dialyzed against 0.01 M glycine buffer pH 8.5. The cytochrome, about 500 mg, now had an iron content of 0.41 % and $E^{1\,\%}_{1\,cm}$ 550 mμ = 20.9. It was now placed on a column of CM–W cellulose (1.5 × 15 cm) prepared according to Peterson and Sober [2], buffered with 0.01 M glycine of pH 8.5. On chromatographing cytochrome c with this buffer and 0.01 M glycine buffer of pH 10.5 some small, colored fractions separated. The main part was finally eluted with 0.05 M glycine buffer of pH 10.5, dialyzed and concentrated by lyophilization.

During the course of this work Hagihara *et al.*[3] published the crystallization of cytochrome c from yeast, beef and pig. Since they gave no analytical data, except the ratio E_{550}/E_{280}, or any convincing proofs of homogeneity of their material we repeated their procedure to produce beef cytochrome c. Crystals were obtained entirely in agreement with their description. Electrophoresis at pH 7.2 in the large Spinco apparatus * revealed three different peaks of the completely reduced cytochrome, all moving towards the cathode. The main component, comprising about 80 %, was the fastest one.

The cytochrome c prepared according to our method, described above, was now submitted to electrophoresis under the same conditions. It was found to show a similar electrophoretic pattern. The main fraction was isolated electrophoretically. This material crystallized in a few hours from ammonium sulphate and ammonia, whereas the crystallization before electrophoresis took a couple of days, just as described by the Japanese workers. Crystals and supernatant showed practically the same spectrophotometrical data. However, for some reason, the ratio E_{550}/E_{280} does not seem to be a very reliable criterion. The crystals contained 0.435 % Fe. Somewhat higher values for the iron content have been reported [4,5] but traces of non-heme iron cannot be excluded in these cases. The light absorption per dry weight (E_{550}, 1 %, 1 cm) was found to be 22.7.

Our conclusion is that crystallization of cytochrome c does not give homogeneous preparations unless followed by further purification, for example by electrophoresis. This is a common experience in protein chemistry.

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