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A simplified procedure for the preparation of cytochrome oxidase by non-ionic detergent

JACOBS *et al.*[1] have described a procedure for the preparation of highly purified cytochrome oxidase from rat-liver mitochondria using Triton X 114* and Triton X 100* as solubilizing agents. This preparation is free from lipid but requires exogenous phospholipids for maximum activity. SUN AND JACOBS[2] modified this procedure and isolated cytochrome oxidase from beef-heart mitochondria with essentially the same properties. This report described a procedure for the isolation of a lipid-containing cytochrome oxidase preparation from beef-heart mitochondria using only Triton X 100 as solubilizing agent. This method yields a highly purified but very active oxidase preparation with considerably less work involved than in most procedures described in the literature.

Beef-heart mitochondria, isolated by the method of LÖW AND VALLIN[3] were suspended in 0.25 M sucrose containing 0.01 M potassium phosphate (pH 7.4) and subjected to sonic irradiation (Bronson Sonifer, maximum power output) for 3 min. After centrifugation at 30000 $\times$ *g* for 30 min, the turbid supernatant and the dark brownish red residue were all discarded. The middle yellowish fraction was collected and resuspended in sucrose–phosphate buffer, homogenized and the protein concentration was adjusted to 30 mg/ml. Enough 10 % aqueous solution of Triton X 100 (w/v) was added, with vigorous stirring, to achieve the final concentration of 1 mg per mg of protein. Solid potassium chloride was then added to the final concentration of 1 M. The mixture was allowed to stand for 10 min at 0° and centrifuged at 40000 rev./min for 30 min in a Spinco preparative ultracentrifuge. A distinct separation into red and green fractions occurred and the red supernatant was poured out. The green residue was collected, resuspended in sucrose–phosphate buffer and the protein concentration was adjusted to 20 mg/ml. Enough aqueous Triton X 100 was added again to the suspension to make 1 mg/mg protein and potassium chloride to 1 M. The mixture was centrifuged at 40000 rev./min for 30 min and the green residue fraction was collected and resuspended. The second treatment caused some loss of the oxidase but was necessary to free the final preparation from any cytochromes *b* or c_1.

Essentially the same treatment was used to solubilize cytochrome oxidase from the second residue. The protein concentration of the suspension was adjusted to 30 mg/ml 10 % aqueous Triton X 100 was added to make 1.5 mg/mg of protein and solid potassium chloride to 1 M. The mixture was allowed to stand for 30 min at 0° and centrifuged at 40000 rev./min for 30 min. The dark brown residue was discarded and the green supernatant fraction was collected and stored at −20°. The final preparation is optically clear and quite stable at this temperature. However, aggregation does occur if the enzyme is heated at 37° for 15 min.

This method for preparation of purified cytochrome oxidase was considerably easier than most of the previous methods[4–6]. If beef-heart mitochondria are available, the entire operation can be finished in 5 h time.

The activities of this enzyme preparation are listed in Table I. Good activity

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can be obtained without phospholipids, but maximal activity can only be obtained after preincubation with phospholipid before assay. Non-ionic detergents of the Tween or Emasol series can replace phospholipids in stimulating the oxidase activity. As indicated in Table I, these activities are identical or higher than most of the cytochrome oxidase preparations described in the literature.

TABLE I

PROPERTIES OF CYTOCHROME OXIDASES PREPARED BY NON-IONIC DETERGENT

Preparation	*Heme a** *content*	*Phospholipid content* (%)	*Specific** activity without phospholipids*	*Specific activity with phospholipids*
Mitochondria	0.97	26	3.49	5.87
Cytochrome oxidase prepared by this procedure	8.2	22.2	56.31	73.3
Lipid-free cytochrome oxidase prepared according to ref. 2	8.9	2	5.52	58.29

* Heme *a* contents are expressed in mμmoles/mg protein.

** Cytochrome oxidase was assayed according to the method of SMITH[7] in citrate–phosphate buffer (pH 6.0). The initial reduced cytochrome *c* concentration was 15 μM. The activities are expressed in μmoles cytochrome *c* oxidized per mg per min.

The visible spectra of this oxidase preparation is identical to that reported by the authors for the lipid-deficient preparation and also identical to that reported previously[4–6]. Chemical analyses demonstrated that this enzyme contained the same amount of heme *a*, as the lipid-deficient preparation. The phospholipid content was substantially higher, close to 20 % per protein basis. The yield is about 50 mg/g of starting sonicated mitochondria.

The solubility of the enzyme depends on the presence of inorganic salts. Removal of the salt by dialysis against 3 % aqueous Triton X 100 caused complete aggregation

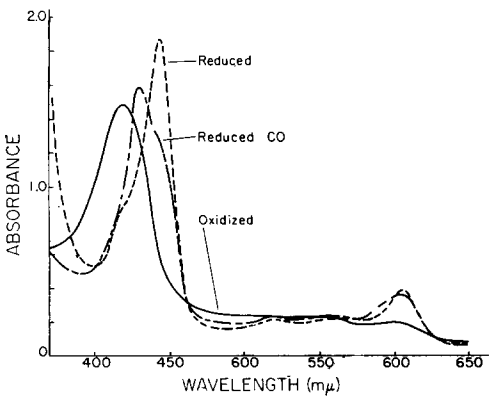

Fig. 1. Visible spectra of purified cytochrome oxidase. Enzyme was dissolved in 0.1 M phosphate buffer (pH 7.4) containing 1 % Triton X 100 with protein concentration 1.8 mg/ml. ———, oxidized; – – –, reduced with sodium dithionite; – – – –, carbon monoxide-treated after reduction with sodium dithionite.

of the enzyme. The aggregated enzyme was not active, but the activity could be regained by solubilizing the aggregated enzyme in 1 % Triton X 100 containing 1 M potassium chloride.

We feel that this cytochrome oxidase preparation is quite significant because it permits a study of the effects of detergent and salt separately. Most cytochrome oxidase preparations described in the literature are solubilized with bile salts, which precludes this advantage. In addition the simple procedures yield a high purity preparation in good yield in a minimum of time.

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