DISRUPTION OF MITOCHONDRIA AND SOLUBILIZATION OF CYTOCHROME OXIDASE BY A SYNTHETIC ZEOLITE

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We report a new method using a synthetic zeolite to disrupt mitochondria from Candida yeast and also beef heart muscle, with concomitant solubilization of the cytochrome complex and other mitochondrial components. The zeolite-solubilized oxidase from the yeast used, remains in the supernatant liquid after centrifugation at 144,000 x g for two hours, as a water-clear, homogeneous phase. However, most zeolite-treated oxidase-containing preparations from heart muscle sediment between 25,000 - 50,000 x g, although a few preparations have been obtained which do not sediment at 144,000 x g. The characteristic absorption spectrum of cytochrome oxidase is readily demonstrated in the supernatant liquids, as well as good cytochrome oxidase activity. No bile salts, detergents, organic solvents or sonic oscillations are required or used.

MATERIALS AND METHODS: All operations were performed in the cold (4° - 5°C.). Glass redistilled water was used. Type 3A zeolite was from the Linde Co., Tonawanda, New York. This is a synthetic crystalline zeolite ("molecular sieve") with a 3 Å pore size which will admit only substances of 3 Å size, or below. In an aqueous system, the molecule that will enter the 3A zeolite pore structure most readily is water (Breck, Eversole, Milton, Reed, and Thomas, 1956). The zeolite is also a cation-exchanger.

Mitochondria were prepared from 10 grams of frozen-thawed yeast

(Candida utilis) from Lake States Yeast and Chemical Co., Rhinelander, Wisconsin), according to Lamanna and Mallette (1954) and from fresh and frozen-thawed beef heart muscle (Slater and Cleland, 1953). The respective isolated mitochondria were washed three times with water, using centrifugation at 144,000 x g for 30 minutes to recover the mitochondria following each wash. The mitochondrial pellet was then thoroughly drained and put in an ice-cooled stainless steel tube. Approximately 5 grams of zeolite was now added to the mitochondria in small portions with blending. Note: (The zeolite must be absolutely dry before use; the interaction between zeolite and mitochondria is exothermic and requires efficient cooling.) The zeolite-mitochondrial blend was next transferred into approximately 50 ml of cold glass re-distilled water. The aqueous suspension was spun at 1200 x g for 5 minutes and a turbid, opalescent brown to brown-red supernatant liquid recovered. The supernatant liquid was then spun at 144,000 x g for one or two hours. Above the brown or brown-red pellet was a clear, homogeneous supernatant liquid , colored light yellow-green (short light path) or a deeper green (long light path). Above the clear supernatant liquid floated a band of white lipid material, very similar in appearance to the lipid layer obtained following centrifugation of bile salt- or detergent-treated mitochondria. Serial extractions may be performed until solubilization of material has ceased.

Cytochrome oxidase activity was determined at 25°C. by following oxidation of dithionite-reduced cytochrome c at 550 mμ (Wainio, Person, Eichel and Cooperstein, 1951, except that 0.05 M tris buffer, pH 7.4 was used instead of PO_4 buffer). Protein was determined by a modified biuret procedure to eliminate heme contributions (Yonetani, 1961).

RESULTS: Fig.1 shows spectra of solubilized oxidase preparations from Candida utilis mitochondria. Similar results were obtained with heart muscle mitochondria. The lower curve shows the spectrum of superna-

tant following centrifugation at 144,000 x g for 1 hour. Characteristic absorption peaks are present as follows: 600-605 mμ - α peak of cytochrome oxidase; shoulder in 560 mμ region - α peak of cytochrome b; 550 mμ region - α peak of cytochrome c; 530-520 mμ- combined β peaks of b and c components; a slight hump at 480 mμ is also present; 440 mμ region - γ peak of cytochrome oxidase; 430 mμ region - γ peak of cytochrome b. There may be a very slight skewing below 420 mμ belonging to the γ peak of cytochrome c.

The upper curve of Fig. 1 shows the visible spectrum of a similar supernatant following 2 hours centrifugation time. (Note: ten-fold expansion of ordinate values). The cytochrome oxidase α peak at 603 mμ is very distinct as are the α peaks of the b and c components seen in the regions of 560 mμ and 550 mμ respectively. The β peak of cytochrome b appears as a skewing of the tracing in the 530 mμ region, and the β peak of cytochrome c appears at 520 mμ. A hump at 480 mμ is also present (flavoprotein?)

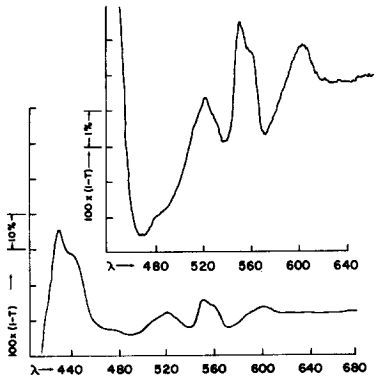

Fig. 1. Direct tracings of recorded ratio spectra of clear supernatant liquid obtained following treatment of Torula utilis mitochondria with zeolite 3A. Dithionite-reduced material in sample cuvette; non-dithionite treated material in reference cuvette. Ordinate scale is in % Absorption, i.e., 100 x (1-T) where $T=I/I_o$.

Representative data to illustrate the cytochrome oxidase activity of such preparations are as follows: The respective final supernatant volumes from the above preparations were 510 and 212 ml. Untreated aliquots of 0.05 ml and 0.2 ml caused 3-minute decreases in absorbancy of reduced cytochrome c (at 550 mμ) of 0.292 and 0.285. The corresponding specific cytochrome oxidase activities of the preparations were 0.087 and 0.070 (μ moles cytochrome c oxidized/min/mg prot). The preparations therefore are of good activity, but low purity. Further purifications are in progress. It should be noted that if 3A zeolite is first moistened slightly by water, subsequent treatment of mitochondria by the moistened zeolite will not effect mitochondrial disruption and concomitant solubilization of cytochrome components. Also, if mitochondria are lyophilized, reconstituted in water or in various buffers, and then washed and packed as described above by centrifugation, mitochondrial disruption and solubilization of cytochromes by zeolite treatment will not occur.

DISCUSSION: An intensive study of mechanisms involved in zeolite induced mitochondrial disruption, and solubilization of cytochrome oxidase is under way in our laboratory. We have also found that zeolites will readily disrupt intact, whole yeast cells (our starting material). Amongst relevant properties of the 3A zeolite used in these studies, is a strong affinity for water. Indeed, zeolites of this family have been used to dehydrate n-butanol to ethers and butenes (Price, 1948). The zeolite also combines avidly with water, even at 100°C. (Breck et al., 1956). At the outset, one must therefore consider that removal of water from mitochondria by the zeolite is one of the events associated with mitochondrial disruption and concomitant solubilization of cytochrome components. Support for this hypothesis is the fact that

slight moistening of the zeolite with water prior to addition to mitochondria, rendered the zeolite ineffective in regard to mitochondrial disruption and cytochrome solubilization. (Breck et.al., 1956, showed that slight moistening of this class of zeolites with water profoundly depressed subsequent reactivity of the zeolite with polar substances.) Also, lyophilization of mitochondria rendered them non-susceptible to the disrupting effects of the zeolite (following mitochondrial re-suspension in, and centrifugation from, water).

The white lipid layer above the clear cytochrome-containing supernatant liquid obtained following high speed centrifugation of zeolite treated mitochondria, is evidence that rupture of lipid bonds also accompanies water removal from, and disruption of, the mitochondria. In this connection it is pertinent that the lipid and protein moietes of a lipoprotein may be held together by water molecules (Hanahan, 1960). We are also considering the possibility that the water linkages involved in the phenomena described above may involve what Bernal (1959) has called "cryohydric" bonds. Finally, we wish to emphasize that the water and lipid interrelationships alluded to above, are part of but do not embody a complete explanation of the phenomena of mitochondrial disruption and cytochrome solubilization by the zeolite, since other properties of the zeolite appear to be involved as well. A fuller experimental report is in preparation.

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