RESTORATION OF ATP-INDUCED CONTRACTION OF PRE-TREATED MITOCHONDRIA BY "CONTRACTILE PROTEIN"

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Ohnishi and Ohnishi (1962 a) have recently isolated from liver mitochondria a protein characterized by an ATPase activity and by physical properties similar to those of the actomyosin of muscle (Ohnishi & Ohnishi 1962 b). They suggested that this "contractile protein" constitutes part of a mechano-enzyme system in mitochondria which could be responsible for the swelling-contraction cycle occurring in these particles (Lehninger 1962 a,b).

It appeared desirable to repeat the experiments of Ohnishi and Ohnishi and to determine if the "contractile protein" possessed a specific activity in the mitochondrial swelling-contraction cycle. Experiments reported in this paper show that the protein fraction extracted from rabbit- or rat liver mitochondria according the procedure of Ohnishi and Ohnishi (1962 a) possesses some but not all of the properties of skeletal muscle actomyosin. However, this protein fraction was found to restore the ATP-induced contraction in mitochondria which had lost that property either after extraction by 0.6 M KCl or after ageing at 0° in isotonic sucrose.

EXPERIMENTAL: Rat liver mitochondria were isolated according to Hogeboom (1955). Rabbit liver mitochondria were prepared in the same way except that 20 mg. of pronase (California Corporation) were added per 10 g. of rabbit liver homogenate. The protein fraction designated as "contractile

protein" by Ohnishi and Ohnishi was extracted from mitochondria of rat liver or rabbit liver and purified according to their procedure. The mitochondria from 100 g. of fresh liver were treated with 100 ml of 0.6 M KCl-0.01 M Tris pH 7.6 for 2 hours at 1°. The saline extract after clarification was diluted 6 fold with distilled water and the pH was adjusted to 6.3. Sometimes, instead of being diluted, the extracts were dialyzed overnight against 5 volumes of water at 1°. Under these conditions a precipitate appeared which was collected by centrifugation. This precipitate was dissolved in 0.6 M KCl; it corresponded to the crude "contractile protein" of Ohnishi and Ohnishi and is designated here as Fraction A. The supernatant fluid which was obtained in the last step and which consisted of proteins soluble at low ionic strength was concentrated by freeze-drying to 5 ml and dialyzed overnight against one liter of distilled water. The precipitate which was formed was removed by centrifugation and the supernatant fraction used for further tests; the latter fraction will be referred to as Fraction B. The water uptake and extrusion by mitochondria corresponding to the swelling and contraction phases were usually measured optically at 520 mμ (Lehninger et al. 1959) and occasionally by gravimetry (Price, Fonnesu & Davies 1956).

RESULTS AND DISCUSSION: In agreement with Ohnishi and Ohnishi, formation of fibers could be observed when a concentrated solution of the Fraction A was injected into a solution of 0.05 M KCl, 0.005 M $MgCl_2$. Addition of ATP led to an apparent shrinking of the fibers. However no change of viscosity (measured with an Ostwald viscosimeter at 21°) was observed when ATP was added to a solution of the "contractile protein" under the conditions given by Ohnishi. In the presence of 10^{-3} M Mg^{++} or Ca^{++}, a small ATPase activity was detected (0.01 μmole P_i released/mg protein/min at 30° and pH 7.3). These results, although in partial agreement with those of Ohnishi and Ohnishi, do not yet provide compelling evidence that this mitochondrial protein fraction closely resembles actomyosin in all respects.

Since rat liver mitochondria extracted by 0.6 M KCl and then allowed to swell under a variety of conditions were found to have lost capacity to contract upon addition of ATP + BSA + Mg^{++}, the protein fractions A and B prepared from rat liver mitochondria were tested for ability to restore mitochondrial contraction. Tests were also carried out in mitochondria pre-aged for several hours or several days at 2^{o} in 0.25 M sucrose, since such mitochondria also lose their ability to contract upon addition of ATP + Mg^{++}. Both protein fractions A and B, when added to either KCl-extracted mitochondria or pre-aged mitochondria, were able to restore to an appreciable extent the capacity of the mitochondria to shrink after addition of ATP, BSA and Mg^{++} (Fig. 1). Only partial restoration could be observed if the extracts were added at the end of the swelling phase rather than at the beginning.

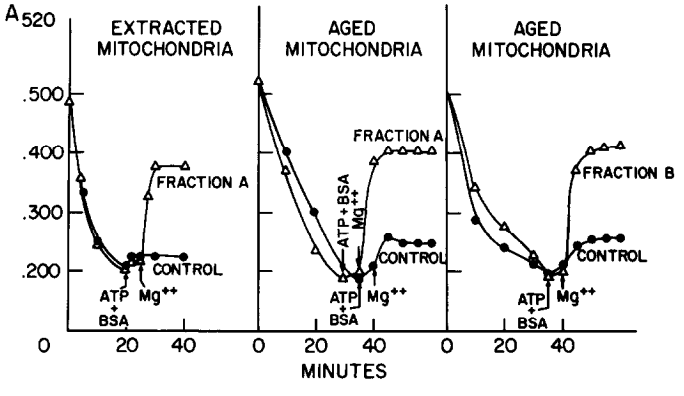

Fig. 1: Effect of the "contractile protein" (Fraction A) and the soluble protein fraction (Fraction B) on contraction of mitochondria
Incubation mixture: 0.125 M KCl, 0.02 M Tris pH 7.4, oleate 3.10^{-6} M (expt. 1), 10^{-5} M (expt. 2 & 3) in a final volume of 3 ml. Mitochondria were previously extracted for 2 hrs. in 0.6 M KCl and resuspended in 0.25 M sucrose after centrifugation or "pre-aged" for 6 hrs. in 0.25 M sucrose. A small inoculum of the mitochondrial suspension (380-500 µg protein) in 0.050 ml of 0.25 M sucrose was added to the medium supplemented or not with Fraction A (260 µg protein) or Fraction B (500 µg protein). ATP (5mM final conc.) + BSA (2 mg/ml fin. conc.) were added at the end of the swelling phase (first arrow), then 5 min. later, $MgCl_2$ (3mM fin. conc.) was added (second arrow). Temp. 25^{o}.

While the shrinking properties of mitochondria over a large range of pH were lost after saline extraction, they were more particularly altered at pH below 7.6 after preageing in 0.25 M sucrose. However, in both cases Fractions A and B were able to restore the ATP-induced contraction to about the same extent. They could restore contraction of mitochondria swollen spontaneously or in the presence of swelling agents such as oleate, Ca^{++}, thyroxine, phosphate. They were without effect when swelling was initiated by glutathione, suggesting that they are different from the factor(s) lost by mitochondria exposed to glutathione (Lehninger & Gotterer 1960; Neubert, Wojtczak and Lehninger 1963). Restoration of half-maximum contraction was found to require about the same quantity of Fraction A as of Fraction B, namely about 200 µg protein when the amount of mitochondria used contained about 400 µg protein. Another property common to both fractions is that their activity is not lost by heating at 100° for 5 min. However their activity is completely lost after extraction of their lipid components by chloroform-methanol (Folch, Lees & Sloane-Stanley 1957). A partial and transitory reconstitution of the

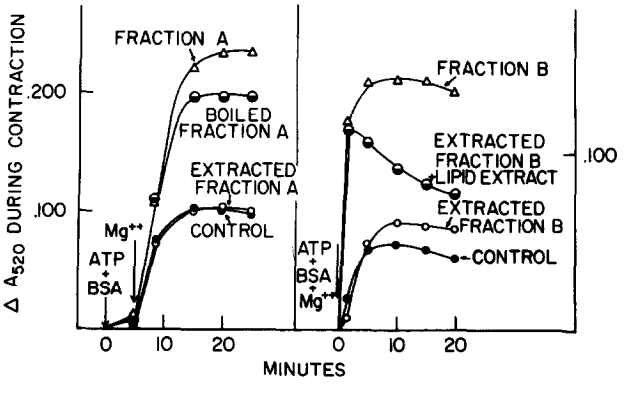

Fig. 2: Effect of boiling or of chloroform - methanol extraction on the ability of the protein fractions (A & B) to restore ATP-induced contraction. Same conditions of incubation as in Fig. 1 - Oleate 10^{-5} M pH 7.4 Mitochondria "pre-aged" for one day. Fraction A: 260 µg protein (expt. 1), Fraction B: 700 µg protein (expt. 2).

initial activity was obtained when the lipid extract was added back to the extracted protein fraction (Fig. 2). An active protein fraction corresponding to Fraction B has also been prepared from digitonin fragments of rat liver (Devlin & Lehninger 1958).

The data presented here thus indicate that during ageing in cold isotonic sucrose or in 0.6 M KCl solutions, rat liver mitochondria release a factor involved in ATP-induced contraction. This factor is associated with a protein fraction (Fraction A) designated as "contractile protein" by Ohnishi and Ohnishi (1962 a) as well as with a second protein fraction soluble in 0.10 M KCl (Fraction B). Both protein fractions restore ATP-linked contraction of aged or extracted rat liver mitochondria. The activity of each fraction is dependent upon lipid components. While the "actomyosin-like" fraction of Ohnishi and Ohnishi requires further chemical and physical characterization, its ability to promote mitochondrial contraction is noteworthy in view of the many similarities in behavior of mitochondrial and myofibrillar ATPases (Lehninger a,b). A following communication will report the identity of the lipid components of mitochondrial protein fractions which possess contraction-restoring activity.

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