THE SOLUBILIZATION AND PROPERTIES OF THE ATPase FROM THE KEILIN-HARTREE HEART MUSCLE PREPARATION

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The Keilin-Hartree heart muscle preparation (HMP) is derived from physically disintegrated mitochondria and contains all of the components for electron transport from succinate or NADH to molecular oxygen. This preparation, like mitochondria, has been extensively used in the study of intracellular respiration. However, in contrast to mitochondria, HMP does not catalyze the phosphorylation during the substrate oxidation and is thus known as a non-phosphorylating particle. On the other hand, since the conceptual formulation and some experimental evidence have indicated multiple steps for the oxidative synthesis of ATP, the term, non-phosphorylating, has become less significant. The knowledge on the extent of the incompleteness of the phosphorylating apparatus in HMP is a prerequisite to the undertaking of the project on the reconstitution of a unified phosphorylating system from non-phosphorylating particles. The present communication briefly reports this facet of the problem: the solubilization of ATPase from HMP; the reconstitution of particulate, oligomycin-sensitive ATPase from the soluble enzyme; and related observations.

METHODS AND MATERIALS

HMP was prepared from beef heart by the centrifugation method as described previously (King, 1961). It was diluted with an equal volume of 0.25 M sucrose and then centrifuged. The pellet was suspended in 0.25 M sucrose. An acetone powder was prepared by slowly mixing 30

volumes of cold acetone with HMP in sucrose medium. The precipitate was collected on a Buchner funnel, thoroughly washed with acetone, and then immediately dried in vacuo. The ATPase activity was determined by either Method I (Myers and Slater, 1957) or Method II (regenerating system with phosphoenolpyruvate, (Pullman et al., 1960)). Oligomycin was a generous gift from Professor H. A. Lardy.

RESULTS AND DISCUSSION

Particulate ATPase in HMP--The role of ATPase in oxidative phosphorylation has been shown conceptually (e.g. Slater, 1953) and experimentally (e.g. Penefsky et al., 1960). Table I indicates the occurrence of ATPase in HMP and inactivation by urea. It can be seen that both the specific activity of the enzyme and its rate of inactivation by urea are practically the same as those reported by Racker (1962) for the particles prepared by a sonic treatment of intact mitochondria. Indeed, calculated from the ATPase activity determined by Method II, HMP contains nearly 20 µg ATPase per mg protein. This computation is based on the assumption that the enzyme reported by Pullman et al., (1961) was the same, with respect to its specific activity, as the ATPase in HMP. The ATPase activity

Table I. The effect of urea on the particulate ATPase activity from HMP

HMP in 0.25 M sucrose containing approximately 8 mg protein per ml, 1.33 M urea, pH 7.4, was treated at 0-4° for the indicated time. The ATPase activity was determined by Method I and expressed in mM x min^{-1} ATP reacted at 1 mg HMP protein per ml. The figures in parenthesis are reported by Racker (1962) for ATPase activity (determined by Method I with a slight modification) in the submitochondrial particles prepared by sonic treatment.

Time (hours)	Activity (mM x min^{-1})
0	0.45 (0.48)
0.5	0.18
1.0	0.11
1.5	0.07
2.0	0.07 (0.07)

determined by Method II was always higher than that by Method I; the reason has been elaborated by Pullman et al., (1960). Many years ago, Myers and Slater (1957) also demonstrated the ATPase activity in HMP from different aims and tactics.

Solubilization of ATPase from HMP--A simple method for the solubilization was developed after examination of numerous other means. It involved the treatment of a mixture consisting of 5 ml glass beads (diameter, about 0.3 mm) in 18 ml 3-5% aqueous suspension of acetone powder of HMP in a Nossal shaker for two minutes. By three successive extractions, a recovery of 40% was usually obtained with a specific activity as high as approximately 2 μmoles ATP reacted per minute (by Method II) per mg protein. The employment of acetone powder as the starting material is similar to that used by Lardy and Wellman (1953) or Selwyn and Chappell (1962). Indeed the latter investigators also used the heart muscle preparation. However, neither group adopted an extraction with a special type of agitation; effective solubilization was realized only through the treatment in the Nossal shaker. After surveying various factors for solubilization, it was found that water was best for the extraction of ATPase from the acetone powder of HMP. An increase of salt concentration greatly decreased the yield. The present investigation was inspired by the method for the fragmentation of intact mitochondria described by Pullman et al., (1958).

Properties of soluble ATPase from HMP--The ATPase activity in the aqueous solution was not sedimented by centrifugation at 144,000 x g for 30 minutes at room temperature. 2,4-Dinitrophenol (DNP) at 0.5 mM increased the activity approximately 50%. As shown in Table II, the activity was relatively unaffected at 30° but diminished at low temperatures. It should be mentioned that the life of the ATPase activity somewhat varied from batch to batch. The stability of the enzyme was greatly affected by the presence of salt, as summarized in Table III. All these properties are again

identical to those reported by Racker (1962) for cold-labile, soluble ATPase or coupling factor I (F_1) isolated directly from intact mitochondria.

Table II. The effect of temperature on the stability of soluble ATPase from HMP

The ATPase activity was determined by Method I in the presence of 0.5 mM DNP. The initial activity was 0.3 mM x min^{-1} at 1 mg protein per ml.

Treatment of samples	% of initial activity remaining
3.0 hours at 30°	97
0.5 hour at 0° then 2.5 hours at 30°	76
1.0 hour at 0° then 2.0 hours at 30°	44
1.5 hour at 0° then 1.5 hours at 30°	35
2.0 hour at 0° then 1.0 hours at 30°	41
3.0 hours at 0°	44

Table III. The effect of salt on the stability of soluble ATPase from HMP

The ATPase activity was determined by Method I. The initial activity was 0.31 and 0.21 mM x min^{-1} at 1 mg protein per ml in the presence and absence of 0.5 mM DNP, respectively. "$t_{30\%}$" is the time required for 30% diminution of the initial activity.

Treatment	% of Activity remaining after 2 hours		$t_{30\%}$ (minutes)	
	-DNP	+DNP	-DNP	+DNP
30°	80	65	220	170
30°, 0.26 M KCl	60	55	160	145
0°	26	22	103	(53)
0°, 0.26 M KCl	0	0	8	7

Reconstitution of the oligomycin-sensitive ATPase--Huijing and Slater (1961) and Selwyn and Chappell (1962) have shown that the ATPase linked with particulate matter, i.e. in the heart muscle preparation, is sensitive to oligomycin whereas the soluble enzyme is insensitive (Pullman et al., 1960). Racker (1962) has succeeded in "conferring", in part, the

oligomycin-sensitivity onto soluble ATPase. We have confirmed and extended these observations with HMP in 0.25 M sucrose, as shown in Table IV. However, the more important demonstration was in the reconstitution of oligomycin-sensitive ATPase from the soluble enzyme and the HMP particle free of ATPase activity as summarized in the second part of Table IV. It was evident that the soluble ATPase was physically reincorporated into the particle. The question of whether urea inactivated ATPase in HMP or urea first caused the dissociation of ATPase from the particle and then inactivated the dissociated enzyme cannot be answered from present results.

Table IV. The effect of oligomycin on ATPase activity and the reincorporation of soluble ATPase into HMP

The ATPase activity was determined by Method II in the absence of DNP. The urea-treated HMP was made by incubating HMP in 0.25 M sucrose and 3.3 M urea at 0° for 5 minutes. The mixture was then centrifuged at 140,000 x g for 20 minutes at 0°, and the pellet was dispersed in 0.25 M sucrose. In the reincorporation sample (No. 9), a mixture of urea-treated HMP and soluble ATPase in the presence of 12 mM $MgCl_2$ was incubated at 30° for 10 minutes. The pellet from centrifugation at 0° was washed once with 0.25 M sucrose and finally suspended in sucrose. Water instead of soluble ATPase was used in the control (No. 10), and no $MgCl_2$ was present in incubation for No. 11.

Sample (final volume = 1 ml)	ATPase activity
	$mM \times min^{-1}$
1. HMP, 0.5 mg in 0.25 M sucrose	0.26
2. (1) + 3 μg oligomycin	0.036
3. Urea-treated HMP, 0.5 mg	0.026
4. (3) + 3 μg oligomycin	0.006
5. Soluble ATPase (from HMP) 0.1 mg	0.25
6. (5) + 6 μg oligomycin	0.27
7. (5) + (3)	0.135 *
8. (7) + 1 μg oligomycin	0.061
9. Pellet from centrifugation of (3)	0.164
10. (9) - ATPase in incubation	0.018
11. (9) - $MgCl_2$ in incubation	0.033
12. (9) + 5 μg oligomycin	0.041

* (cf. also Racker (1962))

These results suggest that HMP does contain a species of ATPase which seems to be identical with that reported by Racker (1962, see also Pullman et al., 1960). The Racker enzyme is, at least in his system, an obligatory component for the oxidative synthesis of ATP (e.g. Pullman et al., 1961).

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