PURIFICATION AND PROPERTIES OF A SOLUBLE MITOCHONDRIAL ATPase FROM CABBAGE, BRASSICA OLERACEA*

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Recently, considerable interest has been expressed concerning the role of the mitochondrial adenosinetriphosphatase (ATPase) in the process of oxidative phosphorylation. Lardy and Wellman (1953), Pullman, et al. (1960), Selwyn and Chappell (1962), and more recently Kettman and King (1963) have described the preparation and properties of soluble ATPase systems from mammalian mitochondria and the obligatory participation of this activity in oxidative phosphorylation in beef heart mitochondria has been demonstrated (Pullman, et al., 1961). There have been, however, no reports in the literature of any similar studies dealing with the corresponding solubilized ATPase from plant mitochondria, although numerous investigations have been carried out with particulate preparations.

In this communication, we wish to report the partial purification and some of the properties of a soluble, magnesium dependent and 2,4-dinitrophenol (DNP)-stimulated ATPase from cabbage mitochondria. Additional results suggest that this activity is closely related to a possible phosphorylated intermediate in the process of oxidative phosphorylation.

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METHODS AND MATERIALS

The mitochondria were prepared from cabbage, Brassica oleracea, by modifications of the procedure described previously (Freebairn and Rammert, 1957). The mitochondrial pellet was suspended in 0.05 M Tris-acetate buffer, pH 6.2 and then added slowly to 10 volumes of cold acetone. The residue was collected and transferred to a Büchner funnel, washed profusely with acetone and finally with dry ethyl ether. All operations with organic solvents were carried out at -18°C. The acetone powder was allowed to dry in the air for several minutes at room temperature and could be stored at -18°C in vacuo at least a month without significant loss in ATPase activity.

RESULTS AND DISCUSSION

Solubilization and Purification of the ATPase. The acetone powder was extracted with n-butanol at -18°C for 24 hours. This treatment appears to remove acetone insoluble phospholipids and greatly facilitates solubilization of the enzyme. The mixture (ca. 5 ml n-butanol/gram acetone powder) was initially ground to a homogeneous consistency with a mortar and pestle and then mechanically stirred for the duration of the extraction period. The residue was centrifuged down, washed several times with dry ethyl ether and then dried as before. Extraction of the n-butanol treated acetone powder was carried out at 0°C with a solution containing 0.05 M Tris-acetate buffer (pH 8.2), 5×10^{-3} M cysteine-HCl, and 10^{-3} M ATP. The extract from 6.0 grams of the n-butanol treated powder was centrifuged at 105,000 xg for 60 minutes at 0°C and then chromatographed on a 2.2 x 60 cm column of DEAE-cellulose. The results are shown in Figure 1. The enzyme after chromatography was approximately 15-fold purified over the ATPase activity present in the whole mitochondria. Prior to carrying out enzymatic assays on the eluant fractions, the striking observation was made that some of the

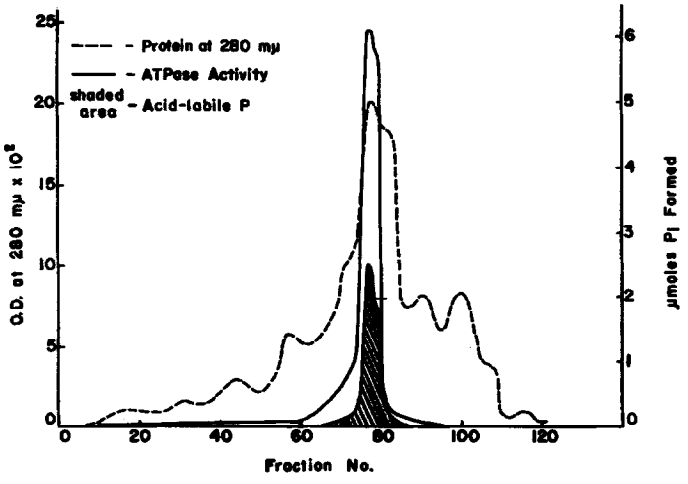

FIGURE 1: DEAE-cellulose chromatography of soluble extracts from n-butanol treated acetone powder from cabbage mitochondria.

Linear gradient elution of the crude extract was carried out at 4°C according to McGilvery (1960) and adapted to a NaCl-Tris-HCl-mercapto-ethanol elution system. The concentration gradient was from 0 to 0.6 M NaCl in 0.01 M Tris-HCl, pH 8.2. The flow rate was 60 ml/hr.

The ATPase activity was associated with an intense yellow coloration that was independent of the presence of flavin. Enzymatic assays were carried out by incubating 1.0 ml aliquots at 30°C for 10 minutes with 1.0 ml of a medium containing 20 μmoles ATP, pH 7.4; 10 μmoles $MgCl_2$; and 200 μmoles Tris-HCl buffer, pH 7.4. The enzymatic reactions were stopped by the addition of 1.0 ml cold 0.8 M TCA to the reaction vessels. ATPase activity was estimated as the amount of P_i formed during the incubation.

P_i assays carried out directly on dialyzed (8 hours, Tris-HCl, pH 7.4) eluant fractions resulted in appearance of acid-labile P_i (shaded curve).

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protein solutions contained a non-dialyzable, acid-labile source of inorganic phosphate which was released in the presence of the acid-ethanol reagent employed in the P_i assay (Martin and Doty, 1949). The results of two experiments were in close agreement and showed that the acid labile P_i appeared only in the fractions that contained the ATPase activity as seen by the shaded area in Figure 1. In light of the report of an ATPase "coupling activity" (Pullman, et al., 1960) required for the

oxidative synthesis of ATP in beef heart mitochondria, it seems reasonable to believe that the present enzyme, possessing ATPase activity and an acid labile phosphate might be an intermediate in the synthesis of ATP.

Properties of the ATPase. The soluble ATPase was found to be markedly stimulated by an ATP regenerating system (Gatt and Racker, 1959). This observation is similar to the findings of Penefsky, et al. (1960) with the soluble ATPase from beef heart mitochondria. Other properties of the enzyme are described in Figure 2 and Table I. A marked difference between the soluble ATPase from cabbage mitochondria and the one

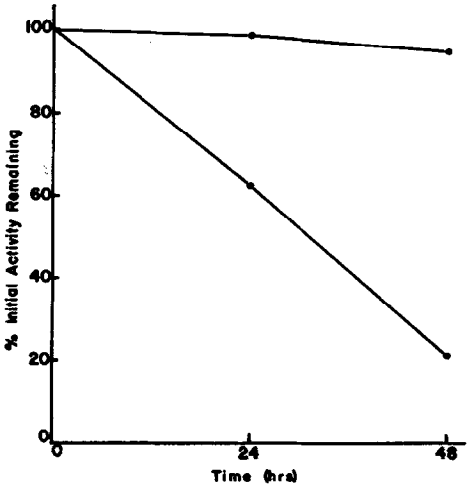

FIGURE 2: The effect of temperature on the stability of the soluble ATPase from cabbage mitochondria.

The enzyme was stored at either 4°C (-●—●-) or 24°C (-o—o-) and then assayed at 24 and 48 hours for ATPase activity. The incubation conditions are the same as those described in Figure 1.

from mammalian mitochondria (Racker, 1962) is the lack of the cold lability property for the plant enzyme as shown in Figure 2. The inhibition of the ATPase by ADP is consistent with the observed

stimulatory effect of the ATP regenerating system, since the rephosphorylation of ADP to ATP would prevent the accumulation of inhibitory levels of ADP.

Table I. The effect of Mg^{++}, DNP and ADP on the soluble ATPase from cabbage mitochondria

The incubation conditions are the same as those described in Figure 1 except that the incubation time was 20 minutes. Enzyme protein was present at a concentration of 0.9 - 1.0 mg/ml.

Experiment No.	Incubation Medium	ATPase Activity (μmoles P_i/mg Protein)
1	Complete	5.82
	" , - Mg^{++}	0.97
	" , + $1x10^{-5}$M DNP	7.12
	" , + $1x10^{-4}$M DNP	6.80
	" , + $5x10^{-4}$M DNP	6.65
2	Complete	6.02
	" , + $3x10^{-3}$M ADP	5.00
	" , + $5x10^{-3}$M ADP	3.65
	" , + $1x10^{-2}$M ADP	1.90

Although Forti (1957) reported the stimulation of ATPase by DNP in a mitochondrial preparation from etiolated pea seedlings, little or no effect of DNP has been observed upon Mg^{++}-dependent ATPase from other plant sources, e.g., whole or fragmented spinach chloroplasts (Baltscheffsky, 1959), whole mitochondria from cabbage (Lotlikar, 1960). The fact that stimulation by DNP is observed with the solubilized ATPase in the present study suggests that either the enzyme exhibits markedly different properties when solubilized or that, when confined to the intact mitochondrion, the ATPase activity is largely masked and that a prerequisite to stimulation by DNP would necessarily require penetration of the latter through the mitochondrial wall. This penetration may occur freely in mammalian mitochondria, thus explaining the observed DNP stimulation.

These results will be followed by a more detailed report in a later publication.

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