SYNTHESIS OF POLYPHOSPHATE BY RAT LIVER MITOCHONDRIA

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The synthesis and hydrolysis of acid insoluble polyphosphates has been observed in many lower species and the enzymes involved have been partially purified by Kornberg and Hughes. Data also exists indicating that in bacteria under experimental conditions of biosynthetic blockade, e.g., nitrogen deficiency etc., polyphosphates accumulate within cells.

Lehninger and others have noted that mammalian mitochondria swell in the presence of mitochondrial substrates and in the absence of ADP but shrink when ATP concentration is maintained at a high level. A reasonable explanation for these observations could be that excess substrate in the absence of adequate concentrations of nucleotide acceptor, activates the turnover of high energy phosphate into a "phosphagen" or polyphosphate pool at a very rapid rate, thus allowing ATP concentration to fall. The data in this report indicate that substrate does markedly stimulate phosphate uptake into a high molecular weight polyphosphate of rat liver mitochondria with a concomitant decrease in ATP concentration.

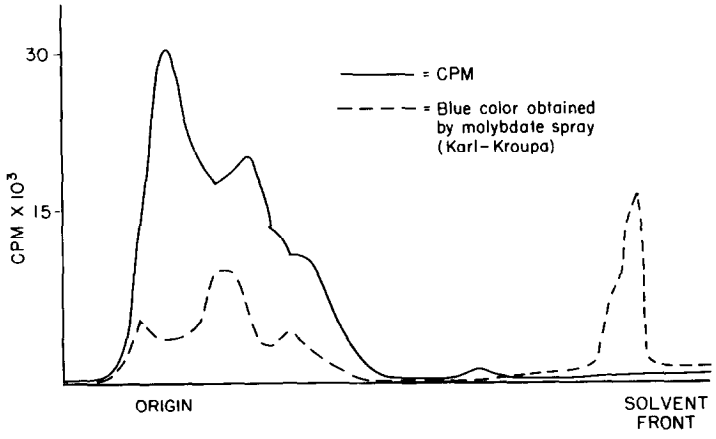
METHOD

P^{32} labeled polyphosphate was isolated, according to the procedure of Katchman. After incubation, mitochondria were sonicated for ten seconds in 4% TCA. The protein precipitate was collected and washed

with cold 5% TCA. It was then boiled in 0.5 N NaOH for 3 minutes. The pH was dropped to 5.5 with acetic acid and protein precipitate removed. The boiling was repeated twice and over 98% of the P^{32} was removed from the protein precipitate. Only negligible amounts of inorganic phosphate were released with boiling. The supernatant was then dialyzed in the cold for several hours at pH 6.5. Polyphosphate could be recovered from this solution by, (1) precipitation with Ba^{++} at pH 4.5, or (2) by separating on Dowex 1 columns by stepwise elution, (the polyphosphate was eluted with 1 M LiCl containing 0.2 N HCl, (Kornberg). The polyphosphate fraction was then put on ascending paper chromatograms, using the acid and basic bidirectional procedure of Karl-Kroupa. After purification, using these procedures, the material contained no carbon or nitrogen and exhibited no ultraviolet absorption. Repeated co-precipitation, by addition of alcohol, of the sodium salt of the radioactive material with synthetic polyphosphate (made according to the procedure of Kornberg) followed by co-chromatography by the two procedures described, revealed that over 50% of the mitochondrial polyphosphate was of large molecular weight, i.e., about 1,000 residues. Polyphosphate was also isolated by dissolving the incubated mitochondria in 7.5 M urea and 0.02 N NaOH (Boyer). Approximately 75% of the high molecular weight polyphosphate was bound to the Dowex, and was eluted with 1 M LiCl. However, the other 25% was still bound to the protein. This fraction could be removed from the protein, after boiling in 0.1 N NaOH for 10 minutes, by another passage over Dowex. Thus, over 90% of the protein-bound Pi^{32} was recovered in the form of polyphosphate. The small fraction remaining on the protein may be in the form of phosphohistidine (Boyer).

RESULTS

Figure I shows the inhomogeneity of the Ba^{++} precipitated material, which had been obtained from the Dowex column.



The effects of substrate and various mitochondrial inhibitors are indicated in Table I. Rat liver mitochondria were isolated according to the procedure of Schneider and Hogeboom using 0.35 M sucrose and 10^{-5} M EDTA. The incubation mixture contained 0.25 M sucrose, 0.02 M Tris, pH 7.4, and Pi^{32}, 5 x 10^{-5} M and the mitochondria obtained from 1/3 of a rat liver (180 gram rat). All additions were made at 0 time. Incubation was for 5 minutes at 30° in air. Mitochondrial size was determined optically (Lehninger) at 520 mμ at the beginning and end of the incubation, using hexokinase and glucose 6-phosphate dehydrogenase. Lipid soluble phosphorus was assayed in counts per minute recovered in washed chloroform-ethanol extract of the TCA precipitated protein (Katchman).

Succinate, a potent swelling agent for mitochondria, markedly increased the incorporation of orthophosphate into polyphosphate, as well as into phospholipid; however, ATP concentration decreased, especially when incubation was prolonged (unpublished). Antimycin, oligomycin, and gramicidin all potentiated whereas DNP and KCN inhibited the effects of succinate, both on polyphosphate and phospholipid turnover.

Thus, rat liver mitochondria, like lower organisms, turnover polyphosphate very rapidly under conditions of excess substrate,

TABLE I.

Effects of Inhibitors on Mitochondrial Polyphosphates, Lipid Pi^{32} Turnover, and ATP Concentration

	Swelling ΔOD x 100	Polyphosphate % Uptake Pi^{32}	Polyphosphate μmoles	Lipid Phosphate % Uptake Pi^{32}	ATP Recovered μmoles
1) Control	6	0.85	.38	0.11	.088
2) Succinate	26	22.0	.34	2.31	.060
3) Succinate + Antimycin	18	42.2	.32	2.56	.051
4) Succinate + Oligomycin	37	31.6	.37	2.24	.022
5) Succinate + Gramicidin	31	28.5	.43	2.21	.056
6) Succinate + DNP	14	12.2	.27	1.05	.023
7) Succinate + KCN	6	14.3	.34	0.59	.026
8) Succinate + ADP + Mg^{++}	15	3.9	.35	-	.47
9) Succinate + EDTA	2	28.3	.38	1.12	.118

Polyphosphate was assayed as the % uptake of added Pi^{32} recovered after dialysis of the alkaline protein extract and colorimetrically after boiling in 1 N HCl for 10 minutes, using method of Berenblum and Chain. The concentration of the additions were as follows: Tris succinate 10^{-3}M, antimycin 2 μg/ml, gramicidin 1 μg/ml, DNP (dinitrophenol) 10^{-4}M, KCN 10^{-4}M, ADP 10^{-3}M, Mg^{++} 10^{-3}M, oligomycin 5 μg/ml, and EDTA (ethylenediaminetetracetic acid) 10^{-4}M.

especially when ATP synthesis is partially blocked (antimycin, oligomycin, and gramicidin). However, when the mitochondria are uncoupled with DNP, polyphosphate turnover is also inhibited. Likewise, when coupling substrates are added (ADP and Mg^{++}), polyphosphate turnover is inhibited. Polyphosphate, therefore, appears to be an alternate, but very active, pathway for labile phosphate metabolism in mitochondria. Some of this polyphosphate is tightly bound to protein, and is not released by passage over Dowex 1 columns (Boyer). Furthermore, swelling

of mitochondria by substrate may be, in part, at least, the result of the shunting of high energy phosphate into the polyphosphate pool and away from the nucleotide pool.

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