A POLYNUCLEOTIDE COENZYME OF OXIDATIVE PHOSPHORYLATION III MECHANISM OF ACTION

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The enzyme system in the bacterium Alcaligenes faecalis that links the formation of ATP to the oxidation of DPNH has been fractionated into three components in this laboratory. One of these is a cytochrome containing particle that catalyzes electron transport without phosphorylation. In order to link phosphorylation to this oxidation, it is necessary to add two other soluble components to the particles several minutes before substrate is added. One of these is destroyed by heat and is called heat labile fraction or HLF. The other is heat stable, and it has been identified as a polynucleotide coenzyme of the RNA type. In crude extracts this coenzyme is tightly bound to the particles. Synthetic polynucleotides containing a single purine or pyrimidine base can replace the natural coenzyme, (4-8). The observation that other polymers could replace the coenzyme was confirmed and extended by the work of Allfrey and Mirsky, who showed that a variety of polyanions could substitute for nucleic acid in this and other reactions of cell nuclei, (1, 2).

These facts suggested that the coenzyme might act in a relatively non-specific way by binding HLF to the particulate fraction. This hypothesis was investigated by incubating mixtures of particles and HLF together in the presence and absence of polynucleotide. The mixtures were then centrifuged and the pellets were tested for phosphorylation activity. If phosphorylation was observed, this indicated that binding had taken place, because, if binding did not occur, the soluble fractions would remain in the supernatant solution. In the control experiment lacking polynucleotide in the mixture before centrifuga-

tion, this component was added to the pellet when it was tested for phosphorylation In a separate test the supernatant solution from this pellet as well as polynucleotide were added to determine whether HLF had remained in the supernatant when polynucleotide was ommitted from the original mixture.

Two methods were used for measuring phosphorylation. The first is a spectrophotometric method which depends on linking TPN reduction to ATP formation with the hexokinase and glucose-6 phosphate dehydrogenase reactions, (8). The second method was carried out under the same conditions except that TPN and G-6-P dehydrogenase were omitted and P^{32} was added. Incorporation of P^{32} into the organic fraction was used to measure phosphorylation following a modification of the method of Berenblum and Chain, (3). DPNH was the substrate in both cases. Preliminary data using the spectrophotometric method to show binding of HLF to the particles by polynucleotide has been published, (10).

A typical experiment using the spectrophotometric assay follows. Two equal mixtures of HLF and particles were made, one of which contained polynucleotide. Both were dialyzed for six hours against a mixture containing .005 M glycylglycine, .01 M KCl, .25 M sucrose at pH 7.4, and IRA 400 in the Cl^- phase. The mixtures were then centrifuged for 20 minutes at 100,000 x G. and the pellets resuspended in .01 M phosphate buffer containing .25% plasma albumin. The pellets were then tested for phosphorylation activity alone and with the additions already outlined. Results are shown in the first half of Table I, from which it can be seen that the pellet formed in the presence of polynucleotide catalyzes more than six times the phosphorylation produced by the pellet formed in its absence. The supernatant solution from this latter pellet produced a marked stimulation when added to it, indicating that in the absence of polynucleotide HLF remained unbound in the supernatant.

Results from one of a series of similar experiments using P^{32} to measure phosphorylation is shown in the second part of Table I. In this experiment dialysis was for one and a half hours against glycylglycine (pH 7.4) and KCl, and 3 μMoles of $MgCl_2$ per ml were added to each mixture before centrifugation.

Here again the pellet formed in the presence of polynucleotide was significantly more active in phosphorylation than the one formed in its absence. Stimulation of phosphorylation by adding back the appropriate supernatant was also more marked in the experiment lacking polynucleotide. These results indicate, in agreement with the first series, that polynucleotide binds HLF to the particulate fraction, and that in the absence of polynucleotide HLF remains in solution. The phosphorylation observed in the pellet lacking added polynucleotide is explained by the fact that HLF was contaminated by polynucleotide in this experiment.

The three components are in the bound form in crude extracts. This is shown by the fact that active pellets can be centrifuged out of these extracts

Table I

Phosphorylation by pellets centrifuged from mixtures of particles and HLF in the presence and absence of polynucleotide

Pellet from mixtures of:	Additions made to pellet: Polynucleotide	Supernatant	Phosphorylation μMoles x 10^3
Spectrophotometric Assay*			
1. Particles HLF Polynucleotide	none	none	59
2. Particles HLF	/	none	9
	/	/ No. 2	31
P^{32} Assay**			
1. Particles HLF	none	none	54 ±4***
Polynucleotide	none	/ No. 1	102 ±8
2. Particles HLF	/	none	22 ±5
	/	/ No. 2	120 ±9

* For details of this assay see reference 8. 0.33 μMole of DPNH was used as substrate.

** Conditions or assay substantially as above, except that P^{32} replaces G-6-P dehydrogenase and TPN. Phosphorylation was measured as the difference in phosphate taken up between a vessel containing 0.6 μMole of DPNH and a control containing an equal amount of DPN.

*** Standard deviation of counting error.

at 100,000 x G. Even after washing in 0.01 M KCl, these pellets show P/O ratios (ATP formed/DPNH oxidized) in some cases approaching or even exceeding 2. Washing in dilute phosphate buffer, however, destroys most of the phosphorylating activity of these pellets.

It seems clear that one function of the polynucleotide coenzyme of oxidative phosphorylation is to bind the heat labile soluble fraction to the particulate electron transporting fraction. The bound complex is relatively easily dissociated, however, and this may provide a physiological mechanism for uncoupling the reaction and thus controlling the rate of oxidative phosphorylation. It also seems quite possible that some of the classic inhibitors of oxidative phosphorylation may act in this way.

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