THE ISOLATION OF POLYNUCLEOTIDE PHOSPHORYLASE FROM ANIMAL TISSUES

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In 1955, Grunberg-Manago and Ochoa [6] were able to isolate from extracts of Azotobacter vinelandii a new enzyme — polynucleotide phosphorylase effecting the synthesis of RNA and similar compounds. In 1956, Kornberg et al. [11] showed that an enzyme was present in soluble extracts of Escherichia coli with the property of catalyzing the reaction of polymerization of desoxyribonucleotides. It has further been shown [1,4,8] that polynycleotide phosphorylase is widely distributed in various bacterial cells, yeasts, spinach leaves and so on.

Numerous attempts have made to obtain polynucleotide phosphorylase from animal tissues. Hilmoe and Heppel [10], for instance, were able to show the presence of adeninepolynucleotide phosphorolysis in the presence of an enzyme isolated from the nuclei of the liver cells of a guinea pig. No synthesis of a polynucleotide was obtained, which, in the authors' opinion, depended on contamination of the extract with nucleases. According to the investigations of other authors [3, 5, 9, 13, 14], incorporation of labeled nucleosidephosphates in polynucleotides takes place in extracts of liver, bone marrow, thymus and ascitic cells on the addition of DNA or RNA. Nevertheless there is no direct proof in the literature of the presence in animal tissues of an enzyme which synthesizes polynucleotides.

The aim of the present investigation was to try to find and isolate from animal tissues an enzyme synthesizing polynucleotides.

EXPERIMENTAL METHOD

As test object we selected regenerating liver, thinking that as a result of the accelerated synthesis of nucleic acids in the process of regeneration, increased polynucleotide phosphorylase activity might be found. Partial hepatectomy (removal of the anterior and left lobes of the liver) was performed in white rats weighing 150-200 g. The rats were sacrificed by decapitation 72 hours after operation, the liver was very speedly extracted, washed with cold 0.14 M NaCl and dried on filter paper, cut into several pieces and placed in acetone with dry ice for 10-15 minutes.

Extraction with 0.15 M KCl was then performed (according to data in the literature, potassium ions activate bacterial enzymes [2]) in the course of homogenization for 3 minutes. The homogenate was centrifuged for 1 hour at a rate of 8000 rpm at 4°. The supernatant fluid was dialyzed in the cold against a phosphate buffer (0.01 M, pH=7.4) for 18 hours with constant mixing. The dialyzate was fractionated with ammonium sulfate in the cold. The precipitates were separated by centrifugation under the same conditions and dissolved in buffered phosphate solution containing cystein (0.01 M, pH=6.8). The solution was dialyzed against the same buffer for 4 hours with stirring, or for 20-24 hours when allowed to stand in the cold. We conventionally called this dialyzate enzyme fraction 1.

TABLE 1

Changes in the Content of Adeninenucleotides and Inorganic Phosphate (P_i) in an Incubation Mixture Containing Enzyme Fractions from the Regenerating Liver of Rats

Enzyme fractions	Temperature of incubation, °C	Incubation time, minutes	Amount of compound, in γ/ml of incubation mixt.		Protein content (in mg) in 1 ml of enzyme	Activity of enzyme, units *
			adeninenucleotides	P_i		
I	0	30	2444,0	200,00	26,65	24,7
I	37	30	1785,2	542,24	26,65	24,7
			—658,8	+342,24		
II	0	30	2483,6	42,48	3,67	57,0
II	37	30	2274,4	129,00	3,67	57,0
			—209,2	+86,52		

* The activity of the enzyme was determined by the fall in the adeninenucleotides in γ/30 min·mg of protein.

TABLE 2

Changes in the Content of Adeninenucleotides, P_i and Readily Hydrolyzed Phosphorus in the Presence of Enzyme Fraction II

No. of enzyme preparation	pH of incubation mixture	Incubation time minutes	In γ/ml of incubation mixture*			Protein content (in mg) in 1 ml of enzyme	Activity of enzyme, units*
			decrease in adenonucleotides	increase in P_i	decrease in readily hydrolyzed P		
1	8,1	15	330,0	50,6	46,6	3,67	90,0
1	8,1	30	209,0	85,0	83,0	3,67	90,0
2	8,2	15	1466,0	34,6		7,82	187,0
2	8,2	60	109,0	85,5		7,82	187,0
3	7,2	15	606,0	24,9	30,3	3,71	163,0

* The numbers given represent the difference between the results of the experimental (incubation at 37°) and control tests (incubation at 0°).

** The activity of the enzyme was determined by the fall in the adeninenucleotides in γ/15 min·mg of protein.

EXPERIMENTAL RESULTS

In preliminary experiments we judged the activity of the enzyme by the increase in inorganic phosphate in the incubation mixture, bearing in mind that synthesis of the polymer from adenosinediphosphates proceeds according to the following scheme [5]:

$$n\text{-}A-P-P \rightleftharpoons (A-P)_n + n\text{-}P_i$$

where A — adenosine and P_i — inorganic phosphate.

Subsequently, however, because of the presence of phosphatase, especially in enzyme fraction I, in addition to determining the inorganic and readily hydrolyzed phosphorus, we measured the quantity of

adeninenucleotides in the acid-soluble fraction obtained by addition of chloric acid to the incubation mixture. As substrate for the reaction we used a preparation of ATP containing 20% of ADP as contaminant. The reaction mixture also contained $MgCl_2$, EDTA (ethylenediaminetetra-acetic acid), glycyl-glycine-tris buffer (pH = 7.2 and 8.2) and the enzyme.

The adeninenucleotides were estimated spectrophotometrically at 290 mμ and 260 mμ, and the inorganic and readily hydrolyzed phosphorus by a colorimetric method (Fiske-Subbarow). In some cases the decrease in the adenine concentration in the acid-soluble fraction after hydrolysis in chloric acid was estimated by the methods of paper chromatography and spectrophotometry.

Further purification of the enzyme was carried out with zinc chloride and ethyl alcohol. To 58 ml of dialyzate, acidified with acetic acid, was added 0.35 ml of 0.5 M $ZnCl_2$. The precipitate was removed by centrifugation in the cold, and to the supernatant fluid was added cold 50% ethyl alcohol, in a volume of 7.7 ml alcohol to 35 ml of centrifugate. After centrifugation for 5 minutes at 8000 rpm in the cold, the residue was dissolved in tris buffer (0.05 M) (enzyme fraction II).

This purification led to an increase in the activity of the enzyme, as determined by the fall in the concentration of adeninenucleotides in the process of incubation, by roughly 2.3 times (Table 1).

By a method described later, we isolated three enzyme preparations, and they all possessed this degree of activity (Table 2).

It will be seen from Table 2 that incubation of the reaction mixture in the presence of enzyme fraction II led to a considerable decrease in the content of adeninenucleotides and readily hydrolyzed phosphorus and to an increase in the inorganic phosphate.

It must be mentioned that during incubation, a decrease in the adeninenucleotide content of the acid-soluble fraction was observed in the first 15 minutes, and as the time of incubation was prolonged, these changes were no longer observed.

This is to some extent in agreement with the findings of Littauer and Kornberg [12], who showed that in the original and insufficiently purified preparations of polynucleotide phosphorylase from E. coli, accumulation of the polymer took place in insignificant amounts, and as the time of incubation was prolonged, synthesis of polynucleotides ceased.

We showed by a special series of control experiments that no changes took place in the test objects in the absence of substrate (ATP and ADP) and also in the absense of enzyme. Heating the enzyme fractions at 100° for 1 minute completely inactivated them. The enzyme preparations were unstable on keeping. On the 7th day of storage at temperatures of 0° and −10°, the activity of the enzyme fractions disappeared.

These afforded only indirect evidence of the possible presence in the different fractions of the regenerating rat liver homogenates of polynucleotide-synthesizing enzymes. In this connection investigations were made in order to detect an increase in the adeninenucleotide content of the acid-insoluble fraction, and thereby to exclude the possible action of adeninenucleotide deaminase. Preliminary experiments showed that enzyme fraction I of regenerating rat liver possesses the power of increasing the content of acid-insoluble product during incubation (by increasing the adeninenucleotides in the acid-insoluble fraction). These findings will be discussed at greater length in subsequent communications.

SUMMARY

The presence of enzyme fractions isolated from the regenerating rat liver gives rise to the following changes during incubation with adenosinephosphates: increase in organic phosphorus content, reduction in easily hydrolyzed phosphorus and adeninenucleoprotein in the acid-soluble fraction and augmentation of the adeninenucleoprotein concentration in the acid-insoluble fraction.

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