EFFECT OF SOME POLYIONS ON METABOLISM OF THE TERMINAL PHOSPHATE OF RIBONUCLEOSIDE-DIPHOSPHATES CATALYZED BY POLYNUCLEOTIDE-PHOSPHORYLASE

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After the discovery of polynucleotide-phosphorylase by Grunberg-Manago and Ochoa [2], many studies of this enzyme have been published. One of the methods used to determine polynucleotide-phosphorylase activity is by the metabolic reaction between inorganic phosphate and the terminal phosphate of ribonucleoside-dishosphates catalyzed by this enzyme [3]. Only scattered details could be found in the literature on the effect of various substances on the velocity of this reaction. Dolin [1], for example, states that the metabolism of $-P^{32}O_4$ with adenosine-5-diphosphate (ADP), catalyzed by an active fraction from Clostridium perfringens, is inhibited by about 40% by protamine sulfate added in a concentration of 80 μg/ml. Littauer and Kornberg [4] reported the stimulant effect of protamine (10 μg/ml) on the metabolic reaction of $-P^{32}O_4$ with ADP catalyzed by polynucleotide-phosphorylase from Escherichia coli. However, with a higher concentration of protein, these authors observed inhibition of the metabolic reaction. Reports have also been published of the influence of certain oligonucleotides on the velocity of the metabolic reaction [5]; inhibition of this reaction by arsenate has also been described [3].

In the present report the authors describe facts demonstrating the effect of certain polyamino acids, protamine sulfate, and spermine on the velocity of metabolism of P^{32}-orthophosphate with different ribonucleoside-diphosphates in the presence of polynucleotide-phosphorylase.

EXPERIMENTAL METHOD

The preparation of polynucleotide-phosphorylase was obtained from E. coli No. 7020 grown on a synthetic medium for 17-18 h at 37° with abundant aeration. The biomass (70-80 g) was collected by centrifugation and broken up by a mechanical disintegrator. The disintegrated biomass was suspended in 4 volumes of 0.05 M Tris-HCl buffer (pH 7.4) and centrifuged at 10,000 g for 20 min. The residue was discarded and the supernatant fluid used for further purification by the method described by Littauer and Kornberg [4].

For the experiment to be described below, a partially purified enzyme preparation of polynucleotide-phosphorylase (fraction ethanol 1) was used; the protein concentration was 64 μg/0.5 ml of incubation mixture. The substrates used included the barium salts of ADP, guanosine-5'-diphosphate (GDP), uridine-5'-diphosphate (UDP), and cytidine-5'-diphosphate (CDP) ("Reanal," Hungary), in the following concentration: ADP and CDP $8 \cdot 10^{-4}$ mole each, GDP and UDP $1 \cdot 10^{-3}$ mole each per sample. The amount of $NaH_2P^{32}O_4$ added to the incubation mixture varied from $3.3 \cdot 10^5$ to $1.7 \cdot 10^6$ pulses/min/μmole. Tris-HCl buffer was added to give a final concentration of 0.2 mole. The concentration of Mg^{++} ions was $4 \cdot 10^{-3}$ M. At the end of incubation at 37°, 0.5 ml of a 5% solution of perchloric acid and 0.1 ml of a 10% aqueous suspension of Norite were added. After standing for 10-15 min in the cold, the Norite was collected by centrifugation and washed 3 times with water in batches of 2.5 ml each, after which the residue was suspended in 0.5 ml of 50% ethanol containing 3 ml concentrated ammonia per liter, and 0.2 ml of the suspension was applied to the target. After drying, the radioactivity was measured on a B-2 apparatus with an end-type counter.

The effect of poly-L-lysine, poly-L-glutamic and poly-L-aspartic acids, protamine sulfate, and spermine on the velocity of the metabolic reaction between P^{32}-orthophosphate and nucleoside-diphosphates was studied.*

* The preparations of poly-L-lysine, poly-L-aspartic and poly-L-glutamic acids, containing 360, 50, and 390 amino-acid residues per molecule respectively, were kindly provided by Professor E. Katchalski (The Weizmann Institute of Science, Rehovoth, Israel), to whom the authors express their gratitude.

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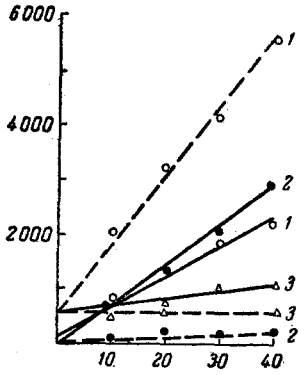

Fig. 1. Effect of poly-L-lysine on metabolism of P^{32}-orthophosphate with the terminal phosphate group ADP (1), UDP (2), and CDP (3). Continuous line—without poly-L-lysine, broken line—poly-L-lysine added. Here and in Figs. 2-4: along the axis of ordinates—in pulses/min/sample, along the axis of abscissas—time of incubation (in minutes).

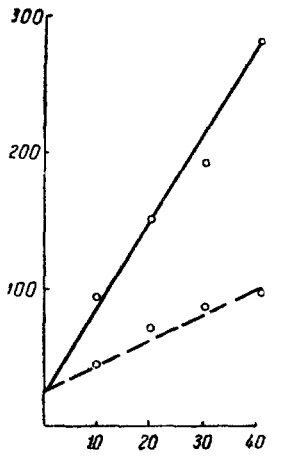

Fig. 2. Effect of poly-L-lysine on metabolism of P^{32}-orthophosphate with terminal phosphate group of GDP. Continuous line—without poly-L-lysine, broken line—poly-L-lysine added.

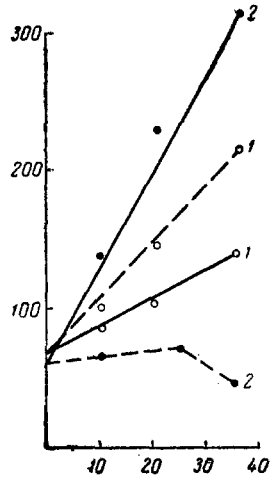

Fig. 3. Effect of protamine sulfate on metabolism of P^{32}-orthophosphate with terminal phosphate group of ADP (1) and UDP (2). Continuous line—without protamine sulfate broken line—protamine sulfate added.

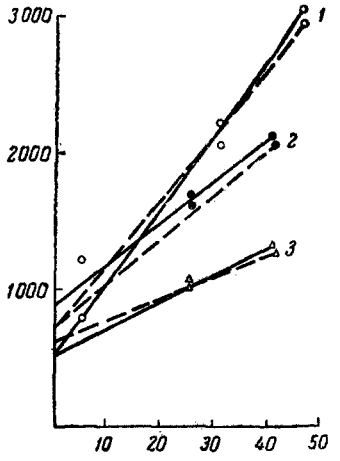

Fig. 4. Effect of poly-L-glutamic acid on metabilism of P^{32}-orthophosphate with terminal phosphate UDP (1) and of poly-L-aspartic acid on metabolism of P^{32}-orthophosphate with GDP (2) and ADP (3). Continuous line—without addition, broken line— corresponding polyamino acids added.

EXPERIMENTAL RESULTS

It is clear from Fig. 1 that poly-L-lysine, when introduced into the system in an amount of 50 μg per sample, increased the metabolism between $-P^{32}O_4$ and ADP by 2.5 times, but practically completely blocked the metabolism with CDP and UDP. The metabolic reaction between $-P^{32}O_4$ and GDP was inhibited by 67% after the addition

of poly-L-lysine in the same concentration (Fig. 2). Protamine sulfate in a dose of 80 μg per sample also increased the velocity of the metabolism of $-P^{32}O_4$ with ADP, but to a lesser degree than poly-L-lysine, and it sharply inhibited the metabolism with UDP (Fig. 3).

When the effect of poly-L-glutamic acid (50 μg per sample) and poly-L-aspartic acid (100 μg per sample) was studied no appreciable change was found in the velocity of metabolism of $-P^{32}O_4$ with the different ribonucleotide-diphosphates (Fig. 4). In individual experiments a very slight stimulant effect of spermine (50 μg per sample) was observed on the metabolism of P^{32}-orthophosphate with ADP and UDP (by approximately 20%).

The results described in this communication show that poly-L-lysine stimulates the metabolic reaction of orthophosphate with ADP only and inhibits metabolism with UDP, CDP, and GDP; the other tested polyamino acids (poly-L-aspartic and poly-L-glutamic acids) had no effect. To what extent the influence of poly-L-lysine is specific and the nature of its mechanisms are difficult to suggest at the present time. Dolin [1], who studied the effect of polypeptides of basic character on the enzymic formation of polyribonucleotides showed that the synthesis of polyadenylate is activated but the synthesis of polyuridylate and polycytidylate is inhibited by them. So far as the mechanism of activation of polyadenylate synthesis is concerned, Dolin suggests that polypeptides of basic character are possibly essential for maintenance of the active configuration of polynucleotide-phosphorylase in solution. Since a clear parallel is observed between the effect of poly-L-lysine on synthesis of polyribonucleotides and on the metabolic reaction of orthophosphate with ribonucleoside-disphosphates, it may be postulated that the mechanism of the influence of this polycation on both reactions catalyzed by polynucleotide-phosphorylase is identical. At the same time, if Dolin's explanation is accepted, it is difficult to determine the reason for the activation of this reaction with ADP and inhibition of the same reaction with UDP and GDP. Presumably this phenomenon is based on a different mechanism associated with the specificity of the substrate.

SUMMARY

Data are presented which show the influence of certain polyaminoacids, protamine sulfate and spermine on the speed of interchange of P-orthophosphate with different ribonucleoside-diphosphates in the presence of polynucletide-phosphorylase from E. Coli. The data adduced indicate that poly-L-lysine stimulates the metabolic reaction of orthophosphate only with ADP and inhibits interchange with UDP, CDP, and GDP. The other polyaminoacids tested showed no influence.

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