MODIFIED METHOD OF PURIFICATION OF THERMOPHILIC POLYNUCLEOTIDE PHOSPHORYLASE RETAINING ITS PHOSPHOROLYTIC ACTIVITY AFTER IMMOBILIZATION

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Synthetic polyribonucleotides are now used widely both in molecular biology and in medical practice. For example, 'Poludan'-a complex prepared of two homopolymers poly(A) and poly(U) is an antiviral drug, used in ophthalmology. In our laboratory of biopolymers we have had many experiments performed with 'Polyguacil' for some years. This potent interferon inducer is a double--stranded complex of homopolymers poly(G) and poly(C). Thus it is necessary now to synthesize in a large scale not only homopolymers but also copolymers: poly(G, A), poly(C, G), poly(G, I). Polymer molecules, used for preparing various complexes, are synthesized by the bacterial enzyme polynucleotide phosphorylase (PNP), (EC 2.7.7.8). The growth of demands for complexes needs large scale preparations of substrates and enzymes. We propose a simple, with a minimal number of stages method of PNP isolation which was tested for PNP from mesophilic bacteria E. coli and from thermophilic bacteria T. thermophilus. Bacterial cells of the strain NB-8 were kindly supplied by Kaboev O. C. (Laboratory of molecular genetics).

We have used the published method of isolation of bacterial DNA-depended RNA polymerase as a prototype. The method is based on addition extract of synthetic basic polymer polyethyleneimine to the cell, so nucleic acids are precipitated with some enzymes involved in their metabolism. This stage allowed us to discard the cytoplasmic proteins with the solution (supernatant). In the course

of further elutions of the precipitate by solutions with increasing ionic strength it was possible to separate the enzymes according to their affinity to nucleic acids. Then it was enough to use the ion exchange chromatography (DEAE-spheron 1000) and the gel chromatography (Sephacryl S-300) for preparing a PNP of great specific activity free from nucleases and phosphatases.

For the immobilization of PNP we have applied an original method elaborated in our laboratory and used chemically modified macroporous glass. PNP is an enzyme with a unique feature. It is able to synthesize polynucleotides and to destruct natural RNA's and its synthetic analogs in very similar experimental conditions. The last process is known as phosphorolysis. The role of this enzyme in bacterial cells has not been identified yet. In some works it has been noted that after purification PNP remained intact to synthetic function but lost its destructive activity either partly or completely. PNP prepared according to our method preserved both types of activity both in batch and in immobilized form. Phosphorolytic activity was investigated on poly(A) as a substrate for PNP. An increasing amount of acid soluble ADP appearing during the polymer destruction was measured spectrophotometrically.