EFFECT OF VARIOUS INDUCING AGENTS ON DYNAMICS OF POLYNUCLEOTIDE PHOSPHORYLASE ACTIVITY IN LYSOGENIC AND NONLYSOGENIC STRAINS OF *Escherichia coli*

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The dynamics of polynucleotide phosphorylase activity in Escherichia coli K-12 (λ) and E. coli B was studied during irradiation with 640-MeV protons, γ Rays, and UV rays and treatment with mitomycin C. High-energy protons and γ and UV rays, in certain doses, were found to activate the polynucleotide phosphorylase (PNPase) of the bacteria. The increase in enzyme activity was directly dependent on the time of incubation (up to 60 min) after irradiation of the lysogenic and nonlysogenic strains of E. coli or their treatment with the antibiotic. On the basis of the analogy obtained between the dynamics of enzyme activity in the two strains no possible role can be ascribed to PNPase in the mechanism of induced development of temperate bacteriophase λ. It is postulated that PNPase may play a role in the mechanism of postradiation degradation of bacterial RNA.

As well as the known factors disturbing the synthesis and degradation of bacterial RNA, the effect of ionizing and nonionizing radiations on these processes has recently been investigated [5, 10, 11, 13].

The results obtained by these workers have laid the foundations for the study of the enzymic mechanisms of the depolymerizing action of radiation on bacterial RNA. Experiments using a mutant strain of E. coli not containing RNase I have shown that this enzyme does not participate in this process [5, 12]. The possible role of two other enzymes depolymerizing RNA (RNase II and PNPase) is still unknown.

The object of this investigation was to study the dynamics of PNPase activity in lysogenic and nonlysogenic strains of E. coli during irradiation with high-energy protons (HEP), γ rays, and UV rays, as well as during treatment with mitomycin C, an antibiotic with a radiomimetic action.

Lysogenic strain E. coli K-12 λ was chosen because this bacterium has high radiosensitivity [8, 9] and comparison of results obtained with this and the nonlysogenic strain is therefore of fundamental importance. In addition, the possible degree of participation of PNPase in the mechanism of radiation induction and subsequent intracellular development of temperature bacteriophage λ was of considerable interest.

EXPERIMENTAL

Cells of E. coli K-12 (λ) and E. coli B were grown on nutrient agar (pH 7.4) for 18 h at 37°C, washed off with 0.9% KCl solution, sedimented, and suspended in synthetic salt medium M-9.

The concentration of cells intended for irradiation with γ rays and protons was $30 \cdot 10^8$/ml, and the concentration for UV irradiation and treatment with mitomycin C was $(2\text{-}4) \cdot 10^8$ cells/ml.

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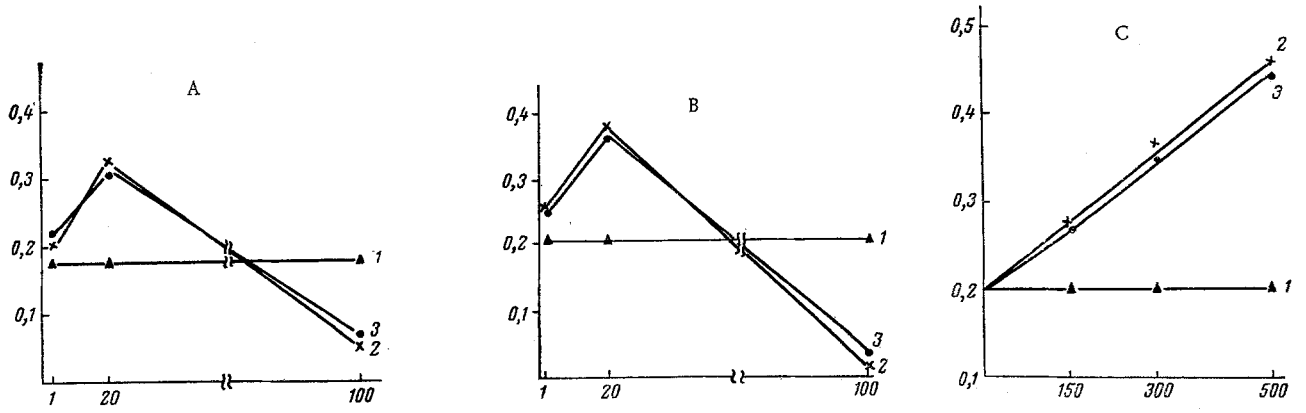

Fig. 1. Character of change in activity of PNPase isolated from lysogenic and nonlysogenic strains of E. coli depending on dose of high-energy protons (A), γ-ray irradiation (B), and UV rays (C). Abscissa – dose of irradiation (A and B – krad, C – ergs/mm^2); ordinate – specific activity of PNPase. 1) Control (unirradiated culture); 2) E. coli K-12 (λ); 3) E. coli B.

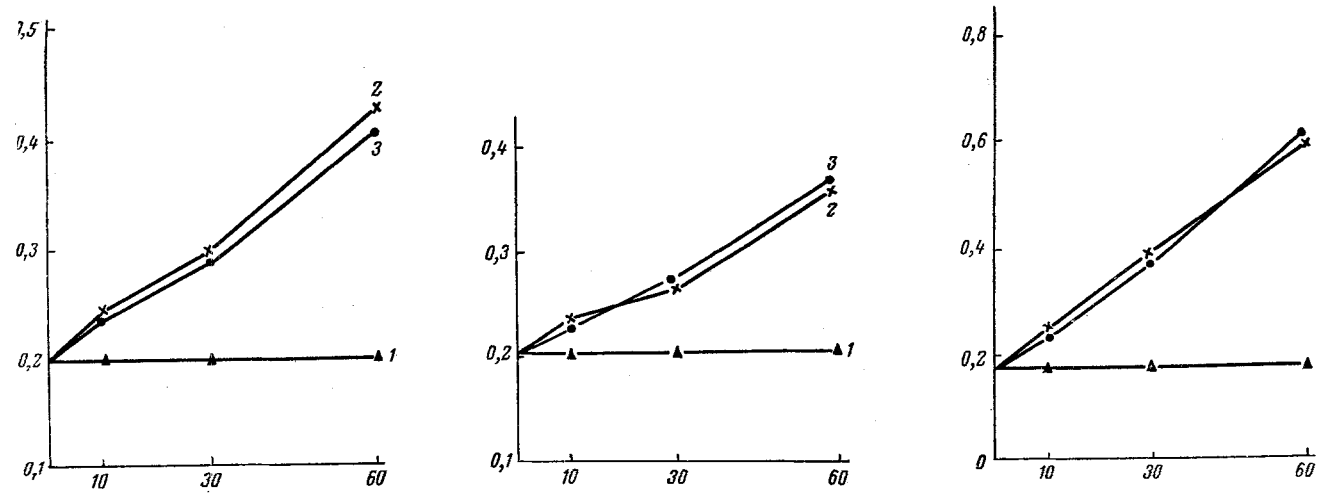

Fig. 2. Effect of γ rays (A), UV rays (B), and mitomycin C (C) on dynamics of activity of PNPase isolated from lysogenic and nonlysogenic strains of E. coli at different time of incubation of bacteria after irradiation or antibiotic treatment. Abscissa – time of incubation (min); ordinate – specific PNPase activity. 1) Control (unirradiated culture); 2) E. coli K-12 (λ); 3) E. coli B.

Irradiation with 640-MeV HEP was carried out in the Laboratory of Nuclear Problems of the Joint Institute for Nuclear Research in Dubno. The dose rate was 30 rad/sec. Dosimetry and irradiation were carried out as described earlier [1]. The source of γ rays (Co^{60}) was the experimental ÉGO-2 γ-ray emitter with dose rate 3.1-3.8 rad/sec. The UV rays were produced by a quartz generator consisting of 4 BUV-15 tubes mounted as described by Mackal et al. [7]. Dosimetry and irradiation were carried out as described earlier [2].

After irradiation and the necessary incubation under optimal conditions (10, 30, and 60 min) the bacteria were collected on the S-44 separating supercentrifuge. The agar layer method of Adams [4] was used to determine the number of lysogenic bacteria induced. The sedimented bacteria were suspended in 0.05 M glycyl-glycine buffer, pH 7.4 (17 g dry weight of bacteria in 68 ml), and broken up by ultrasound at 6-8°C. The broken-up mass was dissolved in 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 used for further purification of the PNPase by the method of Littauer and Kornberg [6].

The partially purified preparation of the enzyme PNPase (the ethanol-I fraction) was used in the experiments. The exchange reaction between P^{32}-labeled inorganic phosphate and the terminal phosphate of ADP was used to determine activity of the enzyme.

The incubation samples contained: $Na_2HP^{32}O_4$ 0.14 mole (from $3.3 \cdot 10^5$ to $1.7 \cdot 10^6$ counts/min/mole); $MgCl_2$ $4 \cdot 10^{-3}$ M; ADP $8 \cdot 10^{-4}$ M; tris-HCl buffer, pH 8.0, $5 \cdot 10^{-3}$ M; PNPase 0.04 ml; proteinconcentration 150 g per 0.5 ml of incubation mixture. At the end of incubation, which lasted 60 min at 37°C, 0.5 ml of 0.5% $HClO_4$ and 0.1 ml of a 10% aqueous suspension of "Norite" activated charcoal were added to each sample. After standing for 20 min in the cold the charcoal was collected by centrifugation and washed 3 times with 0.1 M Na_2HPO_4 solution and once with water. The residue was suspended in 0.5 ml 50% ethanol containing 3 ml concentrated ammonia per liter, and 0.2 ml of the suspension was placed on a platform. After drying, its radioactivity was measured on a BFL-2 instrument with end-window counter.

PNPase activity was determined as the number of micromoles $P^{32}O_4$ incorporated into ADP during incubation for 60 min and calculated per milligram enzyme protein (specific activity).

EXPERIMENTAL RESULTS

The effect of different doses of HEP and γ-ray irradiation on the specific activity of PNPase isolated from these strains of E. coli after incubation for 40-45 min under optimal conditions after irradiation is shown in Fig. 1 (A and B). Analysis of these results showed that the curves of dose versus specific PNPase activity for the two strains of E. coli practically coincide with each other, and that when doses of corpuscular and wave radiation of up to 20 krad were used the increase in PNPase activity was directly proportional to the dose, but a dose of 100 krad led to a sharp decline in enzyme activity. The reason for this behavior is that 100 krad is a knowingly lethal dose causing almost 100% death of the irradiated bacteria.

Despite differences in the radiosensitivity of the lysogenic bacteria and E. coli B, no changes were thus found in the character of the dose – specific activity of PNPase curves for proton and γ-ray irradiation. The activity of the enzyme reached a maximum with a dose of 20 krad.

In the case of UV irradiation (Fig. 1C), however, with doses of up to 500 ergs/mm^2, the PNPase activity rose all the time. It will be noted that ionizing radiation in doses of 16-20 krad showed the greatest inducing activity with respect to temperate bacteriophage λ in the strain E. coli K-12 (λ) used; for UV rays this dose is 250-300 ergs/mm^2 [4]. To study the role of PNPase in the mechanism of induction of bacteriophage λ and its subsequent vegetative development, it was therefore decided to use γ rays in a dose of 20 krad and UV rays in a dose of 300 ergs/mm^2.

PNPase was isolated from samples of lysogenic and nonlysogenic cultures at different times (10, 30, and 60 min) after irradiation or treatment with mitomycin C, incubation being carried out under optimal conditions so that the enzyme activity could be studied at different times during the latent period of intracellular development of the induced bacteriophage.

The experiments with mitomycin were carried out in a similar manner. The dose of antibiotic was 0.5 μg/ml.

The results of these investigations are shown in Fig. 2. Clearly the dynamics of the increase in enzyme activity with the time of incubation after irradiation with HEP or γ and UV rays, and also after treatment with mitomycin C was similar in character for both strains of bacteria. Consequently, the use of ionizing and nonionizing radiation and also of the radiomimetic antibiotic as inducing agents led to no significant difference in the process of induced development of bacteriophage λ as reflected by the test of the increase in PNPase activity. No difference likewise was found with respect to this test between the lysogenic and nonlysogenic strains of E. coli.

These results thus show that HEP, γ rays, and UV rays in certain doses bring about marked activation of PNPase in both lysogenic and nonlysogenic strains of E. coli.

The increase in enzyme activity was found to be directly dependent on the time of incubation after irradiation or mitomycin C treatment of both strains of E. coli within the limits investigated.

The increase in PNPase activity in the case of development of temperate bacteriophage λ induced by the use of radiation and mitomycin is a nonspecific factor for the lysogenic strain and is evidently unconnected with the mechanism of induction and subsequent intracellular development of the bacteriophage. The possibility cannot be ruled out that PNPase is one of the two enzymes which participate in postradiation degradation of bacterial RNA.

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