# EFFECT OF p-AMINOBENZOIC ACID AND AMINOBENZHYDRAZIDE ON DNA REPAIR

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KEY WORDS: injury to the genome; DNA repair; activation of repair; method of screening of repair activators.

There is considerable interest at the present time in the testing of chemical compounds for their ability to activate the DNA repair system in cells. For screening of this sort a model of injury to the genome (usually by γ- or UV-rays or by chemical compounds) is created, after which the rate of its repair is studied when various test substances are added. One aim of the present investigation was to test a different injurious agent for such a screening program, namely heating, for even a slight rise of temperature (to 42-60°C) for a few minutes leads to the appearance of breaks in the bacterial genome [4, 7, 9]. In this case the scheme of screening compounds affecting genome repair may have certain advantages over schemes so far used, for it is harmless, easily reproduced, and can be varied quantitatively under experimental conditions.

As test additives we studied p-aminobenzoic acid (PABA) and its hydrazine derivative — aminobenzhydrazide (ABH). Data on the time course of genome repair after addition of PABA could not be found in the literature although the phenomenon of its effect on repair is known [1, 3]. The reason why ABH was studied is that certain hydrazine compounds are known to be able to restore normal nucleic acid metabolism after the action of a harmful chemical agent [2].

## EXPERIMENTAL METHOD

A culture of *Escherichia coli* M 17 cells, in the stationary phase of growth, resuspended in physiological saline in a concentration of $10 \cdot 10^9$ cells/ml, was used. The samples measuring 0.5 ml were heated to 60°C for 5 min. The samples were then quickly cooled to room temperature and the number of injuries in the genome determined from the value of the double-strandedness factor ($F_{ds}$) of DNA, measured by Rydberg's method [8], with the following modifications: Preliminary introduction of the radioactive label into the nucleoid was not done; the sample was treated with 0.5 ml of lytic solution (0.06 N NaOH, 0.5% sodium laurylsarcosinate in 0.14 M NaCl, pH 9.0-9.2) and incubated in darkness for 10 min at 20°C; after neutralization of the mixture with 2 ml of 0.02 M $NaH_2PO_4$ the samples were treated with ultrasound (22 kHz, 15 sec, 0.3 A. on the UZDN-1 instrument); DNA was analyzed by chromatography on hydroxyapatite [6]; the DNA concentration in the resulting fractions of single- and double-stranded nucleic acids was determined by a fluorometric method, using 4',6-diamidino-2-phenylindole [5]. The value of $F_{ds}$, as a criterion of the integrity of the genome, was calculated by the equation:

$$F_{ds} = \frac{C_{ds}}{C_{ds} + C_{ss}},$$

where $C_{ds}$ and $C_{ss}$ represent the concentrations of double- and single-stranded DNA, respectively, in the fractions obtained chromatographically.

The test substances were dissolved in 0.14 M NaCl, and 25 μl of the solution was added to the samples immediately before heating. The course of DNA repair after heating was judged from the time course of $F_{ds}$ of the genome during the 90 min after the beginning of the last incubation of the cells at room temperature in physiological saline with or without addition of the test substances. Samples containing the original culture and cells also incubated for 90 min but not heated served as controls.

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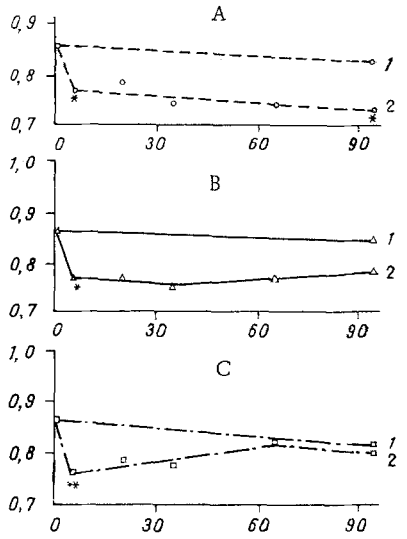


Fig. 1. Effect of addition of PABA and ABH on restoration of integrity of *E. coli* M 17 cell genome after heating. Abscissa, duration of heating (0-5 min) and subsequent incubation (5-95 min) of bacteria; ordinate, value of $F_{ds}$ of DNA. 1) Control culture (incubation at 22°C); 2) experimental culture (heating + incubation at 22°C). A) Incubation without additions, B) with addition of $10^{-5}$ N ABH, C) with addition of $10^{-5}$ M PABA. Mean results of five series of experiments shown. *) $P < 0.05$ compared with control values.

## EXPERIMENTAL RESULTS

It was found in the preliminary stage of the experiments that addition of the test substances in concentrations of between $2 \cdot 10^{-4}$ and $10^{-5}$ M had an approximately equal effect on the magnitude of the changes in $F_{ds}$ after heating of the bacteria, whereas addition in concentrations of $10^{-6}$ M had no effect. Accordingly, in the subsequent experiments PABA and ABH were added to the culture up to a final concentration of $10^{-5}$ M.

Determination of $F_{ds}$ of the cell DNA immediately after heating showed that the integrity of the genome was reduced as a result of such treatment approximately equally irrespective of whether the substances had been added or not (Fig. 1A-C). Consequently, neither PABA nor ABH can prevent injury to DNA induced by heating, and it can thus be postulated that these compounds are not thermoprotectors.

A study of the time course of changes in $F_{ds}$ of the genome after heating of the bacteria gave the following results. The value of $F_{ds}$ in the first (control) version, when physiological saline was added, continued to fall during subsequent incubation of the cells, i.e., the number of injuries in the genome increased (Fig. 1A). In the second version, after addition of PABA, an increase in the value of $F_{ds}$ of the genome was observed during incubation: The value of this parameter rose to the control levels by the 60th minute of culture (Fig. 1C). Consequently, the number of injuries in the heated cells fell fairly rapidly in this case. In the third version, with addition of ABH, no sharp rise in the value of $F_{ds}$ was observed for DNA of the heated cells, just as in the case of PABA, but by the 90th minute of incubation the number of injuries in the genome of the experimental culture did not differ significantly from the corresponding parameter in the control cells (Fig. 1B).

The experiments thus showed that by the use of the technique described above, which is simple, safe, and comparatively rapid, the effects of chemical compounds on repair can be studied. It was shown by the use of the suggested scheme for the screening of substances to be added to the culture medium of bacteria that PABA and ADH cannot prevent injuries to DNA caused by heating. During incubation of *E. coli* M 17 without any additives the cells cannot repair their injured genome, whereas addition of PABA and ABH promotes repair of DNA. PABA stimulates DNA repair more actively than ABH. It can also be concluded from the results that differences found in the ability of PABA and ABH to affect the DNA repair system can be used for the direct study of the mechanisms of action and pathways of synthesis of repair activators and also in microbiology, gerontology, and tumor chemotherapy.

LITERATURE CITED

1. S. V. Vasil'eva, L. S. Davnichenko, E. V. Lutskova, et al., Dokl. Akad. Nauk SSSR, 247, 226, 1979.
2. L. A. Voronova, S. D. Ivanov, and M. A. Zabezhinskii, Vopr. Onkol., No. 2, 58 (1980).
3. I. A. Rapoport, S. V. Vasil'eva, and L. S. Davnichenko, Dokl. Akad. Nauk SSSR, 247, 231 (1979).
4. M. H. E. Andrew and J. P. Greaves, J. Gen. Microbiol., 111, 239 (1979).
5. C. F. Brunk, K. C. Jones, and T. W. James, Anal. Biochem., 92, 497 (1979).
6. K. Erixon and G. Ahnström, Mutat. Res., 59, 257 (1979).
7. C. Pauling and L. A. Beck, J. Gen. Microbiol., 87, 181 (1975).
8. B. Rydberg, Radiat. Res., 61, 274 (1975).
9. S. G. Sedgwick and B. A. Bridges, J. Gen. Microbiol., 71, 191 (1972).

# EFFECT OF THYMALIN ON THE CYCLIC NUCLEOTIDE SYSTEM IN THE MOUSE SPLEEN

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The ability of humoral factors of the thymus to activate the immunoreactive systems of the body has been demonstrated recently [1, 4, 7, 8]. However, insufficient attention has been paid to the study of the biochemical basis of the mechanism of the biological activity of these substances. Parameters reflecting the state of the cyclic nucleotide system in the tissues following administration of thymus preparations *in vivo* are very interesting in this respect. Considering the universality of functions of the cyclic nucleotides as intracellular mediators, it is natural to expect that definite changes in their concentration will be found under these circumstances.

The object of this investigation was to study the time course of changes in components of the cyclic nucleotide system in the mouse spleen under the influence of the thymus preparation thymalin [5].

## EXPERIMENTAL METHOD

Noninbred albino mice weighing 18-20 g were used. Animals of the experimental group were given thymalin in physiological saline by intraperitoneal injection in a dose of 50 μg/g body weight. Choice of this dose was based on the results of experiments in which its administration led to a marked rise of antibody titers in mice [1]. Animals of the control group received physiological saline. Intact animals constituted a separate group.

There were two series of experiments. In series I there were 115 mice, divided into the three groups mentioned above. The animals were decapitated 1, 3, and 10 days after injection of thymalin. The spleen was quickly removed and weighed and the concentrations of cyclic AMP (cAMP) and GMP (cGMP) in it was determined by means of a cyclic AMP assay kit and cyclic GMP RIA kit (Amersham, England) respectively. The radioactivity of the samples was determined in a Mark II liquid scintillation counter (Nuclear Chicago, USA).

The experiments of series II were carried out on 99 mice, killed 1, 3, and 10 days after injection of thymalin. Activity of adenylate cyclase (AC, [6]) and cyclic AMP phosphodiesterase (PDE, [2]) was determined in the splenic tissue. The results were subjected to statistical analysis by Student's and Wilcoxon–Mann–Whitney tests.

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