EFFECT OF PLASMID pKM 101 ON MUTABILITY OF Salmonella STRAINS UNDER THE INFLUENCE OF POLYCYCLIC AROMATIC HYDROCARBONS

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When testing the action of direct mutagens on Salmonella of Ames' strains [2] we showed that the effect of plasmid pKM 101 on induced mutability of bacteria depends on the type of agents relative to whose action the effect of this plasmid is being analyzed. The particular feature of the action of the plasmid relative to changes in the effect of the mutagens is expressed as various types of mutations (frame-shift or base-pair substitutions), the possibility of induction of which is influenced by the plasmid. For instance, the plasmid can induce base-pair substitutions and frame-shift mutations, it can induce base-pair substitutions and increase the frequency of frame shift mutation very slightly, or it can increase the frequency of base-pair substitutions only, without inducing frame-shift mutations.

The object of the present investigation was to study whether this rule extends to mutability of Ames' strains under the influence of mutagens requiring metabolic activation. For this purpose carcinogenic polycyclic hydrocarbons with different levels of carcinogenic activity and their noncarcinogenic analogs were used.

EXPERIMENTAL METHOD

The Salmonella strains were obtained from Dr. Ames [2]. The following polycyclic aromatic hydrocarbons were used: benz(a)pyrene – BP (mol. wt. 252.32), 7,12-dimethylbenz(a)anthracene – DMBA (mol. wt. 256.4), benz(a)anthracene – BA (mol. wt. 228.3), and pyrene. The first three of these agents are carcinogens; pyrene has no carcinogenic activity. The microsomal fraction of rat liver to activate the agents was obtained by Ames' method [3]. To induce enzymes metabolizing the hydrocarbons, male August rats weighing 100-150 g were given an intraperitoneal injection of 20-methylcholanthrene dissolved in vegetable oil, in a dose of 140 mg/kg body weight 48 h before sacrifice. Treatment of the bacteria with the chemicals and seeding to count the number of His^+ revertants per dish were carried out by Ames' method [3]. The mutagenic action of the agents was recorded on the basis of the number of revertants to histidine-independence per dish.

EXPERIMENTAL RESULTS

It will be clear from the data given in Figs. 1 and 2 that the chemical substances tested, which have carcinogenic activity, exhibit a mutagenic action. Significant differences were observed in their mutagenic action relative to plasmid-free strains recording base-pair substitution (strain TA1535) and frame-shift mutations (strain TA1538). In plasmid-free strains BP, DMBA, and BA did not induce base-pair substitutions (strain TA1535) but did induce frame-shift mutations (strain TA1538). During the action of BP on the plasmid-free strain a somewhat greater yield of frame-shift mutants per dish was observed (about 100%) compared with the action of DMBA and BA with maximally effective doses of the agent (Figs. 1 and 2). The stronger mutagenic activity of BP against bacteria not containing the plasmid also was manifested by the fact that low concentrations of the compound were mutagenic (0.1 and 1 μg per dish), although when DMBA and BA were used these doses had no mutagenic action.

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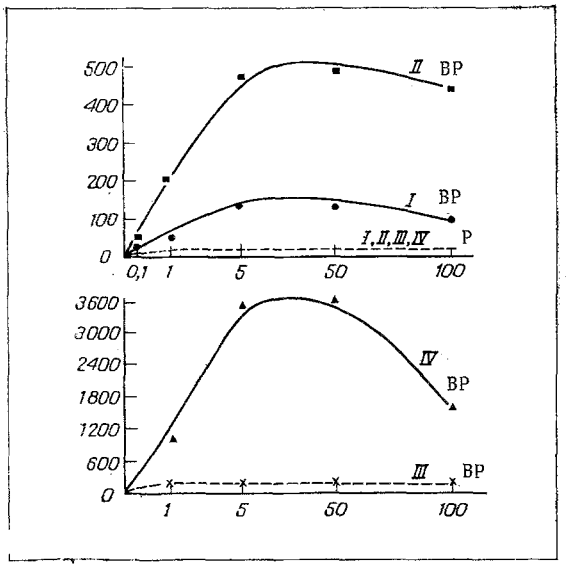

Fig. 1. Mutagenic action of BP on strains of Salmonella. I) Strain TA1538; II) Strain TA98; III) TA1535; IV) Strain TA100. P) Pyrene. Abscissa, dose per dish (in μg); ordinate, number of His^+ revertants per dish.

The mutagenic effect of lower concentrations of BP than of DMBA and BA cannot be ascribed to differences in the molecular weight of the carcinogens, for these differences are very small for BP and DMBA; the molecular weight of BA is lower than that of BP.

As Fig. 1 shows, plasmid pKM 101 considerably increased the yield of frame-shift revertants (strain TA 98) under the influence of BP (the maximal yield of mutants with a dose of 5 μg per dish). To a somewhat lesser degree the plasmid increased induction of frame-shift mutations under the influence of DMBA (Fig. 2). Mutagenesis in the strain containing the plasmid began to be recorded under the influence of DMBA in doses of 1-5 μg per dish. The effect of the plasmid on the mutagenic action of BA was less marked still. The results given in Figs. 1 and 2 show that in the presence of plasmid pKM 101 in Salmonella strains not only are frame-shift mutations induced, but the formation of base-pair substitutions also becomes possible under the influence of carcinogens studied (strain TA100). The greatest mutagenic effect appeared under the influence of BP, during the use of which the mutagenic effect reached a maximum when the dose was 5 μg per dish (Fig. 1).

Maximal induction of base-pair substitutions under the influence of DMBA and BA in the presence of the plasmid was observed when larger doses of the agents were given (from 100 to 500 μg per dish). Meanwhile, comparison of the dose dependence of mutagenesis induced by DMBA and BA shows the following noteworthy feature. The appearance of base-pair substitutions in a strain containing plasmid pKM 101 under the influence of BA began to be recorded at substantially lower doses than those of DMBA. However, when this strain was treated with higher doses of the agents (100 and 500 μg per dish) more marked mutageneisis was observed in bacteria treated with DMBA (Fig. 2).

Pyrene induced neither base-pair substitutions nor frame-shift mutations either in strains with or without the plasmid (Fig. 1).

The carcinogenic polycyclic hydrocarbons tested thus induce only frame-shift mutations and do not induce base-pair substitution in plasmid-free strain of Salmonella; as regards the intensity of their mutagenic activity, these agents can be arranged in the following order: BP > DMBA = BA. The intensity of the plasmid effect as regards both induction of frame-shift mutations under the influence of these agents and base-pair substitutions was clearly defined in the case of BP. The maximal yield of mutants after treatment with high doses of mutagens was observed in the case of the action of DMBA on bacteria containing the plasmid, compared with the same bacteria treated with BA. However, in the presence of the plasmid, BA induces base-pair substitutions in much lower doses than DMBA.

The data showing that the plasmid can induce mutations not caused by the carcinogens tested in the experiments described above in plasmid-free bacteria (base-pair substitutions) are in agreement with earlier

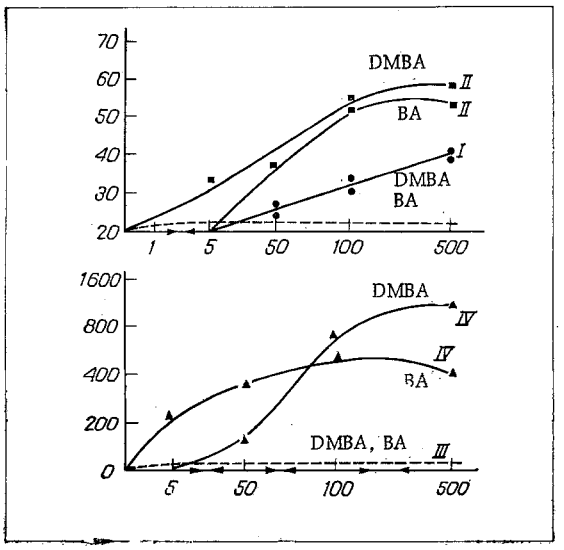

Fig. 2. Mutagenic action of DMBA and BA on strains of Salmonella. Legend as in Fig. 1.

suggestions of the possible unique character of mutagenesis connected with the presence of plasmid [1], and on the need to take account of this unique difference when the potential carcinogens are selected.

According to data described in this paper the plasmid induces an effect only during the action of the carcinogens tested, and not of their noncarcinogenic analog. In the presence of plasmid, BA induces base-pair substitutions in very low doses, which were observed in the experiments with DMBA. Consequently, in experiments with bacteria containing the plasmid high mutagenic activity of a preparation possessing relatively low carcinogenic activity may be observed. It should be pointed out that in experiments with bacteria not possessing the plasmid, mutagenic activity corresponding to carcinogenicity was not observed: Both DMBA and BA induced frame-shift mutations with equal yet low frequency.

Meanwhile, the results show that the intensity of mutagenesis connected with the presence of a plasmid does not correlate with the degree of carcinogenic activity characteristic of the agents tested. This was manifested particularly clearly when the plasmid effect was compared in the case of DMBA, an active carcinogen, and BA, whose carcinogenic activity is very low.

According to the results described above, another fact which deserves attention is that in strains containing the plasmid the carcinogens studied induced not only mutations, which can be induced in plasmid-free bacteria, but also mutations (base-pair substitutions) that do not arise in such bacteria.

These results are evidence of the need to determine more precisely the criteria that must be taken into account when bacterial systems are used in practice for primary screening of carcinogens.

LITERATURE CITED

1. A. G. Skavronskaya et al., Dokl. Akad. Nauk SSSR, 236, 460 (1977).
2. B. N. Ames, F. D. Lee, and W. E. Durston, Proc. Natl. Acad. Sci. USA, 70, 782 (1973).
3. B. N. Ames, J. McCann, and E. Yamasaki, Mutat. Res., 31, 347 (1975).
4. J. McCann, N. E. Spingarn, J. Kobori, et al., Proc. Natl. Acad. Sci. USA, 726, 979 (1975).