DEPENDENCE OF THE FREQUENCY OF SPONTANEOUS AND INDUCED MUTATIONS OF DIFFERENT NATURE ON THE PRESENCE OF PLASMID pKM101

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To increase the sensitivity of strains of salmonellas used for preliminary screening of mutagens (potential carcinogens) the use of plasmid pKM101 has been suggested [9]. The mechanisms lying at the basis of the action of plasmids on bacterial mutability is not yet known, so that the desirability of their use for assessment of agents dangerous to man is questionable [5]. Data showing that plasmid pKM101 can endow salmonellas with the capacity for induced mutagenesis are particularly interesting. This conclusion was reached from experiments with UV light, which is known to act as a carcinogen [8] but, in the absence of plasmids, does not induce mutations in salmonellas [2].

In this investigation the action of UV light and of chemical carcinogens was studied on Ames' strains of salmonellas, by recording the appearance of base pair exchanges and frame-shift mutations in cases when they contain or do not contain plasmid pKM101.

EXPERIMENTAL METHOD

The strains of salmonellas used were obtained from Dr. B. N. Ames [4]. The first pair of strains carries the his G46 mutation (base pair exchange): TA1535(hisG46 rfaΔbiouvrB) and TA100(TA1535/pKM101), the second pair the his D3052 mutation (frame-shift mutation: TA1538(his D3052 rfaΔbiouvrB) and TA98(TA1538/pKM101). A BUF-15 lamp served as the source of UV light. The following chemical mutagens were used: N-methyl-N'-nitro-N-nitrosoguanidine (NG, from Aldrich Chemical Co.), 4-nitroquinoline oxide (4-NQO, from Daiichi Pure Chemicals Co. Ltd.), and ethylmethanesulfonate (EMS, from Serva). The substance with State Registration Number 012074 [3] was obtained from Dr. L. M. Fonshtein. Treatment with 4-NQO and EMS was carried out in 1/15M phosphate buffer, pH 7.0; preparation No. 012074 was dissolved in dimethyl sulfoxide and treatment of bacteria with it was carried out in physiological saline. NG was treated by the method described by Miller [1]. The action of the chemical mutagens was stopped by sedimenting the bacteria and subsequent washing. Histidine-independent revertants were selected on minimal glucose-salt medium [7] with traces of histidine (0.7 μg/ml). The survival rate was determined on the same medium with 20 μg/ml histidine. When the frequency of mutants was counted, a correction was introduced relative to the spontaneous background.

EXPERIMENTAL RESULTS

The data given in Table 1 indicate that UV light does not induce either base pair exchanges or frame-shift mutations in bacteria of plasmid-free strains. In the absence of the plasmid only frame-shift mutations appeared under the influence of 4-NQO and preparation No. 012074, and no base pair exchanges were induced; NG and EMS induced base pair exchanges with a high frequency, but did not induce frame-shift mutations (Table 1).

Plasmid pKM101, which increases UV resistance, endows bacteria with the ability to form UV-induced base pair exchanges (strain TA100) and frame-shift mutations (strain TA98). Mutants of the first of these

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TABLE 1. Absolute Figures for Yield of Mutations in Experiments with Mutagenic Agents

Mutagen and its dose	TA 1535			TA 100			TA 1538			TA 98		
	1	2	3	1	2	3	1	2	3	1	2	3
UV light, ergs/mm^2												
0	1,6	9	—	0,8	210		1,7	5		3,8	16	
1	1,1	6	—	0,9	347	152	1,5	8	—	4,1	27	3
2,5	1,0	13	—	0,9	527	352	1,5	10	—	3,1	63	15
5	0,7	13	—	0,9	1078	964	1,49	6	—	2,7	111	35
25	0,002	6	—	0,5	2324	4 228	0,5	2	—	2,1	334	151
4-NQO, μg/ml												
0	1,6	13	—	0,9	98		1,6	9		1,4	17	
0,005	1,5	5	—	0,7	188	130	1,8	16	4	1,6	42	16
0,05	0,9	9	—	0,5	300	404	1,9	26	9	1,3	58	31
0,5	0,3	6	—	0,5	2189	4 182	1,0	222	213	0,9	324	341
No. 012074, μg/ml												
0	1,0	8	—	0,8	117		1,7	17		1,4	24	
0,1	0,9	4	—	0,7	129	17	1,1	56	36	1,3	70	35
1	1,1	6	—	0,9	140	26	1,2	253	197	1,1	199	160
10	0,3	4	—	0,4	375	645	1,0	1855	1838	1,7	2023	1176
EMS, %:												
0	2,1	7		0,9	54		1,8	13	—	1,3	16	—
0,1	2,4	62	23	0,6	79	42	1,8	19	—	1,0	18	—
1,0	0,7	3040	4 333	0,14	2010	13 971	0,36	4	—	0,19	9	—
2,0	0,2	2042	10 175	0,1	1700	16 460	0,02	1	—	0,04	2	—
NG, μg/ml												
0	1,1	7		0,6	162		1,2	13	—	1,1	18	—
0,2	1,0	37	30	0,5	233	142	0,4	11	—	0,6	17	—
0,5	1,0	625	618	0,5	958	1 592	—	—	—	—	—	—
2,0	0,5	2806	5 598	0,3	2357	7 317	0,6	22	—	0,5	28	—

Legend. 1) Number of living cells ($\times 10^8$ per dish), 2) number of mutants per dish; 3) frequency of mutants ($\times 10^8$ cells/ml).

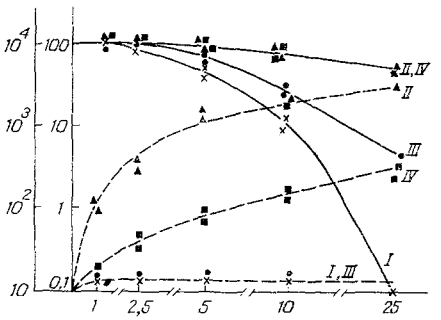

Fig. 1. Mutagenic and inactivating action of UV light (in ergs/mm^2) on strains of S. typhimurium. Here and in Figs. 2 and 3: I) TA1535 (crosses), II) TA100 (triangles), III) TA1538 (circles), IV) TA98 (squares). Continuous lines represent survival rate of cells, broken lines induction of mutants to prototrophism. Ordinate, frequency of mutants per 10 cells (a) and survival rate, in % (b); abscissa, dose of agent.

types were induced with a higher frequency and appeared in response to extremely low doses of UV light (0.5-1.0 erg/mm^2), which do not cause a lethal effect. Within the limits of these low doses of UV light the frequency of UV-induced mutagenesis in strains containing plasmids almost reached a maximum (Fig. 1). During the action of 4-NQO on strains containing plasmids, within the limits of low doses of this agent the frequency of appearance of frame-shift mutations increased considerably. During the action of higher doses (1.0 μg/ml or more) differences in the frequency of induction of frame-shift mutations in the strain containing plasmids and in that without plasmids practically disappeared. In the presence of plasmid pKM101, 4-NQO induced base pair exchanges. Under these circumstances the frequency of appearance of these exchanges was greater than that of frame-shift mutations (Fig. 2A). A similar rule was observed during induction of mutations by substance No. 012074 in strains containing plasmid pKM101. In experiments with this mutagen, compared with

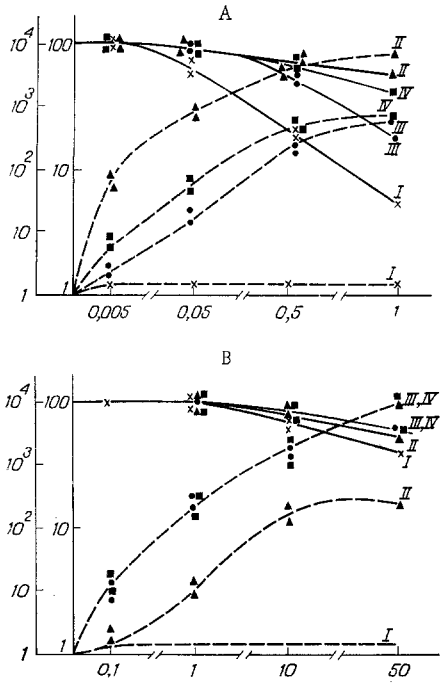

Fig. 2

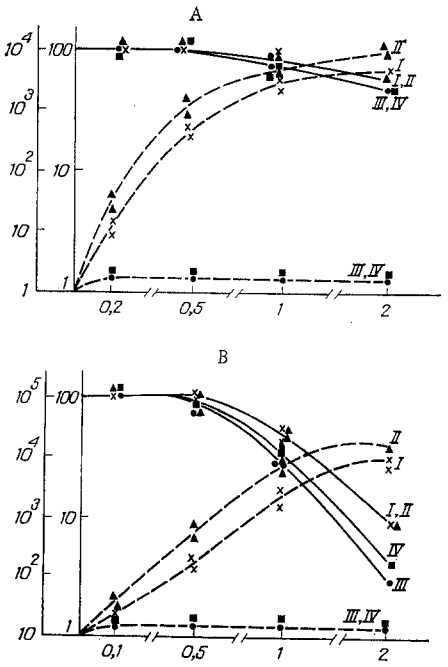

Fig. 3

Fig. 2. Mutagenic and inactivating action of 4-NQO (A, in μg/ml) and of preparation No. 012074 (B, in μg/ml) on strains of S. typhimurium. Duration of treatment with 4-NQO and preparation No. 012074 was 40 min at 37°C.

Fig. 3. Mutagenic and inactivating action of NG (A, in μg/ml) and EMS (B, in%) on strains of S. typhimurium. Duration of treatment with NG 40 min at 37°C, with EMS 60 min at 37°C.

4-NQO, a lower frequency of base pair exchanges was observed than of frame-shift mutations and the increase in the latter under the influence of the plasmid was somewhat smaller (Fig. 2B).

The effect of plasmid pKM101 on the mutagenic action of NG and EMS consisted in a well-marked increase in induction of base pair exchanges. Unlike the pattern regularly observed in the experiments with UV light, under the influence of NG and EMS the plasmid did not affect the ability of the bacteria to produce frame-shift mutations (Fig. 3A, B). Plasmid pKM101 considerably increased the frequency of spontaneous base pair exchanges; against a spontaneous background of frame-shift mutations the plasmid had appreciably less effect (Table 1).

The effect of the plasmid was thus manifested to a greater degree relative to base pair exchanges. This applied to both induced and spontaneous mutagenesis. UV light, of all the agents tested, was unique as regards the effect of the plasmid. During its action the plasmid facilitated the appearance of frame-shift mutations, which was not observed in the case of the action of NG and EMS. Meanwhile the ability to produce base pair exchanges was conferred by the plasmid in the case of the action of all agents tested (UV light, 4-NQO, preparation No. 012074), which do not induce such mutations in plasmid-free strains.

The results of these investigations support the hypothesis expressed previously regarding the replication mechanism of the plasmid effect [2]. This mechanism explains the strongest manifestation of the plasmid effect relative to base pair exchanges. The appearance of ability to form UV-induced frame-shift mutations may be connected with the unique character of injuries induced by UV light (dimers), which may perhaps be realized as frame-shift mutations on replication of DNA with the participation of the plasmid replicator. We know that single dimers inhibit replication [6]. This could account for manifestation of the plasmid effect in response to very low doses of UV light.

On account of the possible unique mechanism of bacterial mutagenesis associated with the presence of plasmids, it must be considered that the solution to the problem of their use for screening mutagens which are potential carcinogens requires further experimental investigation.

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EFFECT OF THIOPHOSPHAMIDE ON THE FREQUENCY OF CHROMOSOMAL ABERRATIONS IN ATAXIA-TELANGIECTASIA HETEROZYGOTES

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Ataxia-telangiectasia (AT), or the Louis–Bar syndrome, is an autosomal-recessive hereditary disease with severe progressive cerebellar ataxia, disturbances of immunity, and telangiectasias in the cornea and skin, and it belongs to the group of syndromes with chromosomal instability. The patients show an increased spontaneous level of chromosomal aberrations [1, 5] and predisposition to malignant neoplasms [8]. On the question of heterozygous carriers of the AT gene a report has been published describing some increase in the spontaneous level of chromosomal aberrations in lymphocyte cultures and, in particular, in fibroblast cultures [7]. Induced mutagenesis has not been studied in these cultures. Considering the fact that persons heterozygous for AT are fairly widespread in the population (about 1%) [6], it was decided to study the principles governing induced mutation in their cells.

The frequency and spectrum of chromosomal aberrations were compared in cultures of lymphocytes from two groups of donors (heterozygotes for AT and subjects of the control group), exposed to different doses of thiophosphamide.

EXPERIMENTAL METHOD

Cultures of peripheral blood lymphocytes from six heterozygous carriers of the AT gene (parents of patients with ataxia-telangiectasia) and from six healthy donors were used. The experiments were carried out within a period of 6 months as the patients were admitted, and one essential condition was observed: simultaneously with the lymphocyte culture of the subject heterozygous for AT, a lymphocyte culture of a control donor was used in the experiment. This was because the true concentration of commercial thiophosphamide

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