REPAIR OF DNA INJURIES INDUCED BY THIOPHOSPHAMIDE IN EMBRYONIC FIBROBLASTS OF 101/H and CBA MICE

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KEY WORDS: repair of DNA injuries; thiophosphamide; inbred mice.

Mice of the 101/H line are highly sensitive to the mutagenic action of thiophosphamide, more so than CBA mice [1], possibly on account of defective repair of induced DNA injuries.

In the investigation described below changes in the molecular mass of single-stranded DNA fragments were studied under the influence of thiophosphamide, and ability of DNA injuries to undergo repair was determined in cells from 101/H and CBA mice.

EXPERIMENTAL METHOD

Primary cultures of embryonic fibroblasts from 101/H and CBA mice were used. The cells were cultured in Eagle's medium with the addition of glutamine, antibiotics, and 20% bovine serum. The cells were incubated in medium containing ^{3}H-thymidine (5 μCi/mmole) for 24 h, or in some experiments, in medium containing ^{3}H-methionine, labeled in position 2 (5 μCi/ml), and with ^{14}C-thymidine (2 μCi/ml). The fibroblast cultures were incubated for 30 min with thiophosphamide (final concentration 1 mg/ml) at 37°C, after which the thiophosphamide was washed off and the cells were incubated in fresh medium for 24 h. During this period the survival rate of the cultures, determined from adherence of the monolayer to glass, was unchanged. The molecular mass of the DNA of the embryonic fibroblasts was studied before the action of thiophosphamide (K), immediately after its removal (0), and at various time intervals after removal. The molecular mass of the DNA was determined by a modified method [5]. Samples of 0.1 ml of lytic mixture (1N NaOH, 0.1 M EDTA), 0.1 ml of cell suspension (5×10^4 cells in Hanks' solution), and 0.2 ml of lytic mixture were applied successively to the top of an alkaline sucrose gradient (0.7N NaOH, 0.3 N NaCl, 0.01 M EDTA) in a volume of 4.8 ml. Lysis of the cells was carried out for 2 h at 20°C. The samples were centrifuged on the AH-650 rotor of a Beckman L-2-65 centrifuge at 35,000 rpm for 90 min at 20°C. The gradient was fractionated from the bottom. The radioactivity of the fractions was measured in Triton-toluene scintillator, using a program for conversion into counts per minute against an external standard. The sedimentation coefficient was determined by the method in [3]. The molecular mass of DNA was calculated as described in [5]. The results were subjected to statistical analysis by Student's t test.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that immediately and 30 min after removal of the thiophosphamide the molecular mass of the DNA in cells of 101/H and CBA mice showed no appreciable change compared with the control values ($0.01 < P < 0.05$ and $P > 0.05$ respectively for lines 101/H and CBA). In the course of subsequent incubation there was a strong increase in the sedimentation rate, which reached a maximum after 4-6 h (Table 1, Figs. 1 and 2). Under ordinary conditions of centrifugation (35,000 rpm, 90 min) most of the DNA at that time consisted of a rapidly sedimenting complex, located on the bottom of the gradient. The formation of DNA-DNA and DNA-protein cross-linkages may lead to an increase in the sedimentation rate of DNA; the DNA-protein linkages may be protein bridges linking together the DNA strands [4]. The present investigations showed that the ratio of radioactivity of the protein level (^{3}H-methionine) to the radioactivity of DNA (^{14}C-thymidine) in the fraction of rapidly sedimenting DNA was not increased but, on the contrary, it was reduced compared with the control

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TABLE 1. Changes in Molecular Mass of Single-Stranded DNA Fragments from Embryonic Fibroblasts of CBA and 101/H Mice during Incubation after Exposure for 30 min with Thiophosphamide

Incubation time, h	Number of experiments		Molecular mass (M ± m)			
	line CBA	line 101/H	line CBA	line 101/H	line CBA	line 101/H
K	11	15	(1,37±0,04) 10	(9,32±0,37) 10^7		
0	4	4	(1,36±0,05) 10^8	(1,22±0,11) 10^8	1,05	1,2
0,5	3	3	(1,37±0,07) 10^8	(1,20±0,10) 10^8	1,06	1,3
2	3	3	(1,96±0,02) 10^8	(1,76±0,12) 10^8	1,6	1,9
	6	6	(2,24±0,14) 10^8	(2,76±0,46) 10^8	1,9	3,1
4	4	2	(1,39±0,26) 10^9	(1,39±0,11) 10^9	12,2	15,5
6	2	2	(1,93±0,02) 10^8	(1,87±0,12) 10^8	1,8	2,3
	1	1	8,37.10^8	8,37.10^8	6,9	13,1
12	3	3	(1,52±0,01) 10^8	(1,61±0,05) 10^8	1,4	2
20	1	1	1,49.10^8	1,68.10^8	1,2	1,6
24	3	3	(1,28±0,18) 10^8	(1,27±0,11) 10^8	1,03	1,4

Legend. Ratio experiment/control determined using control for that given experiment.

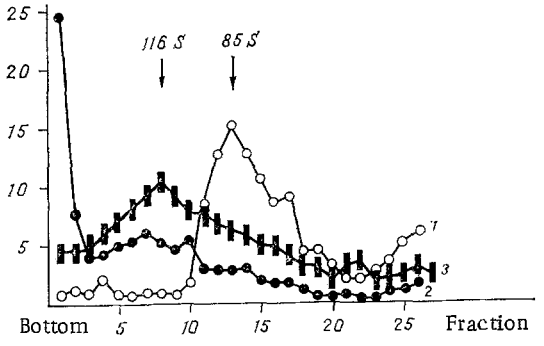

Fig. 1

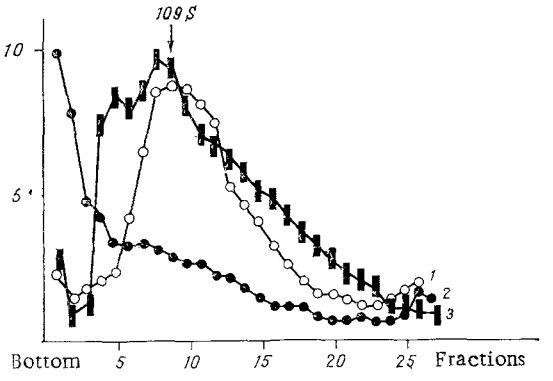

Fig. 2

Fig. 1. Changes in molecular mass of DNA of embryonic fibroblast of 101/H mice during incubation after exposure for 30 min with thiophosphamide (1 mg/ml). Sedimentation profiles of DNA in alkaline sucrose gradient: 1) control; 2) after incubation for 4 h; 3) after incubation for 12 h. Abscissa, Nos. of fractions of gradient; ordinate, percentage of total radioactivity.

Fig. 2. Changes in molecular mass of DNA of embryonic fibroblasts of CBA mice during incubation after exposure for 30 min with thiophosphamide (1 mg/ml). Legend as to Fig. 1.

(1.78 and 4.08 respectively). Consequently, if thiophosphamide, like other alkylating agents [2, 4], also induces the formation of DNA-protein cross-linkages, the relative contribution of these injuries to the formation of a rapidly sedimenting complex evidently cannot be great. The cause of the marked increase in the sedimentation rate was probably DNA cross-linkages between strands.

To determine Svedberg's coefficient of the rapidly sedimenting complex, centrifugation was carried out at slower speeds and for a shorter time (26,000 rpm, 80 min). Under these conditions two DNA fractions were discovered: those sedimenting less rapidly and more rapidly. The ratio between the two fractions varied in different experiments, evidently due to the stochastic character of formation of cross-linkages between the different regions of DNA. The molecular mass of DNA in the two fractions for the given time points (4.6 h) was similar for cells from mice of lines 101/H and CBA ($P > 0.05$ for the two fractions). The permeability of the cell membranes for thiophosphamide, metabolism of the compound in the cell, interaction with the target molecule, and other processes leading to the appearance of a rapidly sedimenting complex probably followed a similar course in cells of the two lines of mice.

During prolonged incubation of the cells (up to 12 h or more) a decrease in the sedimentation rate of DNA was observed after removal of the thiophosphamide. This may be evidence that mouse cells can repair DNA injuries induced by thiophosphamide. Restoration of the molecular mass of DNA took place more rapidly in cells of the CBA mice than in those of the 101/H mice. Maximal differences in the degree of repair of DNA injuries between these lines were found 12 h after removal of the thiophosphamide (Table 1, Figs. 1 and 2). At that time the molecular mass of DNA in the CBA cells was close to the control, whereas in the 101/H cells it was twice as high as the control. The molecular mass of DNA of fibroblasts of the CBA mice after 24 h had virtually reached the control value ($P > 0.05$), but in the 101/H mice the values of this parameter differed significantly in the experiment and control ($P < 0.01$). Repair of DNA cross-linkages in cells of 101/H mice thus takes place much less efficiently than in cells of CBA mice.

The data are evidence that DNA injuries induced by thiophosphamide appear and increase in the course of time. The sites of injury are evidently DNA cross-linkages between strands. Mouse cells also were found to be capable of repairing DNA injuries induced by thiophosphamide, and differences were found in the rate of repair of DNA cross-linkages between 101/H and CBA mice. These results, together with evidence of the relatively high sensitivity of mice of 101/H line to the mutagenic action of thiophosphamide, point to the presence of effective repair of thiophosphamide-induced DNA injuries in the cells of this line of mice.

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ABILITY OF CONJUGATIVE PLASMIDS OF SEROLOGICALLY TYPED *E. coli* TO INHIBIT GENETIC TRANSFER FUNCTIONS OF DEREPRESSED Flac AND pAP22-4 PLASMIDS

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In the classification of the various plasmids detectable in natural bacterial populations, determination of their ability to inhibit genetic transfer function (Tra function) of the F plasmid of *E. coli* K-12 is widely used [10, 11]. This property (the Fin^+ character) is probably controlled by different genetic systems of plasmids, which determine the synthesis of specific protein inhibitors, depressing the functions of transfer genes (tra genes) of the F plasmid [8].

To assess the functional properties of these systems, the inhibitory properties of a group of plasmids identified previously [1, 4-6] by the writer in cells of serologically typed strains of *E. coli* isolated from man and from livestock were investigated with respect to genetic transfer functions of the Flac plasmid (a substituted variant of the F plasmid), and also of the F-like plasmid pAP22-4, derepressed for these functions and discovered by the writer in cells of strain AP1 [2], and marked by incorporation of the transposon Tn1 in its structure [5].

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