ダイズ試験系におけるガンマー線誘発突然変異に対するベンズアントラセンの修飾効果

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Modulating effect of dimethylbenzanthracene on gamma-ray mutagenesis in the soybean test system

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ABSTRACT

Dimethylbenzanthracene (DMBA), a strong tumor initiator, did not show mutagenic activity in the soybean test system either alone or in combination with tumor promoter, TPA. In combination treatments with DMBA and gamma-rays, the mutagenicity of gamma-rays was not affected by post-treatment with DMBA. However, DMBA pre-treatment clearly reduced gamma-ray induced spotting frequency, suggesting that DMBA affects mutagenesis in gamma-irradiated soybean cells.

1. INTRODUCTION

Contamination of land, water and air with a variety of environmental pollutants is now a common phenomenon, and may result in physiological as well as genetic damage to living organisms. Studies of mutagenic activity of pollutants in higher plants are relevant because of their importance as food sources and because plants cannot escape from their growing site and may, therefore, be directly attacked by these substances. Reports of contamination of food staffs by DDT or benz(a)pyrene show that carcinogenic chemicals can be incorporated into plant tissues (Sandermann 1982). It may be assumed that contamination of plants by environmental pollutants will increase in the future due to the expansion of industrial or biotechnological activities. With this view in mind, we have been examining the genetic effects of some chemicals, including carcinogenic substances, by using the soybean test system. (Fujii 1980; Fujii 1981; Fujii and Inoue 1983; Inoue *et al.* 1986).

9, 10-dimethyl-1, 2-benzanthracene (DMBA) is a strong tumor initiator in mouse-skin carcinogenesis; a remarkable incidence of tumors has been observed with this drug in combination with 12-*o*-tetradecanoylphorbol-13-acetate (TPA), a well known tumor promoter (Hecker 1967; van Duuren 1969). The mutagenic activity of DMBA is widely recognized with cultured mammalian or microbial assay systems (Hollstein *et al.* 1979). The widespread occurrence of carcinogenic hydrocarbons in the environment and the current social awareness of the hazardous effects of many environmental contami-

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nants have prompted numerous investigations into the mechanism of action for this hydrocarbon (Jerina *et al.* 1977). In this paper we report on the absence of mutagenic activity of DMBA, and a modulating effect of this chemical on gamma-ray induced mutagenesis in the soybean test system.

2. MATERIALS AND METHODS

Chemicals: DMBA was purchased from Wako Pure Chemical Ltd., Tokyo. One hundred mg of DMBA were initially dissolved in 10 ml dimethylsulfoxide (DMSO), and then diluted with distilled water to give required concentrations.

Gamma-rays: Gamma-rays were delivered from a source of 2.22 GBq ^{137}Cs at an intensity of 4.99 Gy/hr.

Mutation test: Strain T-219 of the soybean, *Glycine max*, was used for the mutation test. The Y_{11} gene in this strain is incompletely dominant; therefore, several kinds of genetic and/or chromosomal abnormalities caused by chromosomal structural changes, somatic crossing-over, or forward and backward mutations can be detected by the appearance of variously colored spots on leaves of heterozygotic $Y_{11}y_{11}$ plants (Vig 1975; Vig 1982). Each experiment was started with 25 g of dry seeds, or about 130 seeds. The seeds were soaked for 24 hr at 20°C in 30 ml of diluted DMSO or in the same volume of solutions containing various amounts of DMBA. Then, depending on the experiment, either air-dried or soaked seeds were exposed to gamma-rays.

Treated seeds were sown in soil in a greenhouse for several weeks and the mutation frequency was measured by the average number of mutated spots per leaf. Two simple leaves at the stage of full expansion were chosen for counting spots.

3. RESULTS

Effect of DMBA

Because DMSO was used as a solvent and diluted further with distilled water, 5% DMSO in an equivalent volume for the highest DMBA concentration was used as the control treatment. No increase in mutation spots was observed at 10 to 100 μg/ml of DMBA (data not shown) or at 0.25 to 4 mg/ml (Table 1). However, some abnormal growth or deformed leaf development was observed with the treatment at 4 mg/ml. It was concluded that DMBA alone had no mutagenic activity in the present test system.

Effects of combination treatment with TPA

Since the combination treatment of DMBA with TPA exhibited a strong tumor incidence on mouse skin carcinogenesis (Hecker 1967; van Duuren 1969), the effect of combination treatments of these two chemicals on soybean

Table 1. *Types and frequencies of spots observed on two simple leaves of soybean T-219 after treatment with DMBA*

DMBA Concentration (mg/ml)	No. of leaves examined	Number of spots per leaf			
		Yl*	DG*	Db*	Total
DMSO (5%)	130	0.6	0.4	0.3	1.3
0.25	121	0.6	0.5	0.2	1.3
0.5	127	0.5	0.6	0.2	1.3
1.0	120	0.5	0.5	0.4	1.4
2.0	117	0.6	0.4	0.4	1.4
4.0	79	0.6	0.5	0.3	1.4

* Yl, yellow; DG, dark green; Db, double.

mutagenesis was investigated. Seeds pre-treated with DMBA (100 μg/ml to 2 mg/ml for 6–24 hr) were treated with 25, 50 or 100 μg/ml of TPA solution for another 24 hr. An increase in spotting frequency could not be detected for any combination treatments (data not shown). An absence of mutagenic activity of TPA in the soybean test system was reported in our previous experiment (Fujii *et al.* 1983).

Effect of DMBA in combination with gamma-rays

To investigate the effects of DMBA on gamma-ray mutagenesis, combination treatments with DMBA and gamma-rays were undertaken. In general, combination treatments of mutagenic chemicals with radiation have shown a synergistic effect for mutation induction (Yamaguchi *et al.* 1975). This ex-

Table 2. *Frequencies of spots observed on two simple leaves of soybean T-219 after combination treatments with gamma-rays and DMBA*

Treatments	No. of leaves observed	Total No. of spots per leaf
—	107	1.0
Gamma-rays 5.4 Gy+H_2O	87	11.7
Gamma-rays 5.4 Gy+1 mg/ml DMBA	107	11.3
Gamma-rays 5.4 Gy+2 mg/ml DMBA	93	11.5
H_2O +Gamma-rays 1.1 Gy	104	9.9
1 mg/ml DMBA+Gamma-rays 1.1 Gy	113	5.7
2 mg/ml DMBA+Gamma-rays 1.1 Gy	81	3.8
—	86	0.8
2 mg/ml DMBA	93	0.8
H_2O +Gamma-rays 1.6 Gy	75	15.9
1 mg/ml DMBA+Gamma-rays 1.6 Gy	67	6.3
2 mg/ml DMBA+Gamma-rays 1.6 Gy	59	3.2

periment was divided into 2 parts: in Experiment 1, seeds were treated first with 5.4 Gy of gamma-rays and then with DMBA: In Experiment 2, seeds were treated first with DMBA and then with 1.1 or 1.6 Gy of gamma-rays.

In Experiment 1, the mutagenicity of gamma-rays was not affected by post-treatment with DMBA (Table 2). Replicate experiments with the same gamma-ray doses and drug concentrations consistently gave the same results (data not shown). In Experiment 2, 1.1 and 1.6 Gy radiation doses were used because of a much higher radiosensitivity for pre-soaked seeds than for air-dried seeds. DMBA pre-treatment clearly reduced the frequency of gamma-ray induced spotting, and the higher concentration (2 mg/ml) was more effective than the lower concentration (1 mg/ml) (Table 2). Frequencies for three types of spots, which result from forward (Y1) and backward (DG) mutations, and somatic crossing-over (Db) (Vig 1975; Vig 1982), were all reduced with DMBA pre-treatment (Fig. 1). These results indicate that three kinds of mutational events responded equally to DMBA pre-treatments.

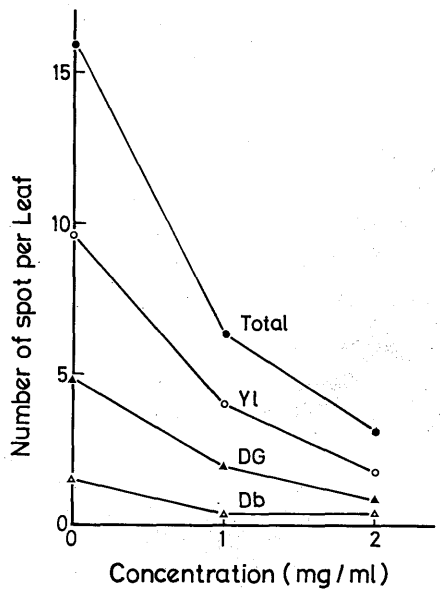

Fig. 1. Decrease of different types of gamma-ray (1.6 Gy) induced mutations with increases in DMBA concentrations. Yl, yellow; DG, dark green; Db, double.

4. DISCUSSION

DMBA showed mutagenic activity in a variety of test systems as reviewed by Hollstein *et al.* (Hollstein *et al.* 1979). This chemical is a more potent carcinogen than benz(a)pyrene and is bound to a higher extent to DNA in the target tissues in mouse skin and rat liver. However, mutagenic activity of DMBA in the Ames test is only about one sixth that of benz(a)pyrene. This

discrepany could be explained if the binding of DMBA to *Salmonella* DNA were lower than that of benz(a)pyrene (Luts 1979). DMBA may not bind well with soybean DNA because no increase of spotting frequency was observed in the present experiment (Table 1).

The mutagenic activity of DMBA was exhibited with metabolic activation by the rat S9 fraction in Chinese hamster V79 cells, *Salmonella* TA97, or *Escherichia coli* K12 (Krahn and Heidelberger 1977; Moreau and Devoret 1977; Sakai *et al.* 1985), or by plant extract in *Salmonella* TA98 (Pankova *et al.* 1986). Recent investigations indicated that plant metabolism efficiently convert promutagens to mutagenic products (Higashi *et al.* 1981; Plewa 1978; Scott *et al.* 1978; Takehisa and Kanaya 1983). Metabolic activation *in vitro* with S9 preparations of several plant species including *Helianthus, Nicotiana, Zea, Tradescantia* resulted in the activated compounds inducing mutations in microbial test systems (Gentile *et al.* 1985). Krahan and Heidelberger (1977) suggested that variation in activating potential depended on inducer-specific qualitative and quantitative differences in metabolism. An absence of activity of DMBA in the present experiment may suggest an absence of a metabolic pathway which activates DMBA to mutagenic status in our soybean test system.

In the combination treatment with DMBA and gamma-rays, a remarkable decline due to DMBA pre-treatment of mutational events induced by gamma-rays was observed (Table 2 and Fig. 1). No such decline in mutation frequency was observed with DMBA post-treatment. One of the possible explanations for this phenomenon is DMBA induced repair function which effectively eliminated DNA damages caused by gamma-rays.

Although the repair mechanism(s) or biological reaction(s) which caused the mutation frequency decline by DMBA pre-treatment is obscure, our results suggests that repair system(s) involved in different organisms may vary widely. A systematic evaluation of the inherent biologic action of chemicals with plant metabolism and repair of genetic damage with metabolic states in plant material is necessary for a better understanding of mutagenesis in plant cells.

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