Acta Biochimica Polonica
Vol. 40 No. 1 1993

The ability of thiram to inhibit eukaryotic topoisomerase II and to damage DNA

Iwonna Rahden-Staroń, Hanna Czeczot and Barbara Kowalska-Loth*

Department of Biochemistry, Institute of Biopharmacy, Medical School, S. Banacha 1, 02-097 Warsaw, Poland
**Institute of Biochemistry, Warsaw University, Al. Żwirki i Wigury 93, 02-089 Warsaw, Poland*

Thiram (tetramethyl-bis-thiocarbamyl disulphide) (CAS No. 137-26-8) is a subject of interest in genotoxic studies because **i**, it is extensively used as a crop fungicide and industrial antioxidant and **ii**, its chemical structure closely resembles that of disulfiram, a drug used in aversion-therapy of chronic alcoholics.

Thiram has been found mutagenic in several bacterial tests [1, 2]. It also induces sperm abnormalities in mice [3] and increases the SCE[1] frequency in human lymphocytes [4]. However, the mechanism of mutagenic and cancerogenic effects of thiram is unknown. Looking for the possible reason of mutagenic effects of thiram we employed numerous bacterial strains which allow to identify changes in DNA introduced by the mutagen. In the previous work we showed that thiram did not methylate DNA in the O-6 position of guanine and did not induce the SOS system in bacteria (in preparation). In this study we have investigated the influence of thiram on the mutation activity of thiram in TA102, a unique strain among the battery of Ames tester strains, which allows to detect oxidative and cross-linking mutagens.

The second, used strain *E. coli fpg*$^-$ mutant, allows to check the possible ring-opening action of thiram. We also present results which point to the inhibitory effect of thiram on eukaryotic topoisomerase II.

The effect of thiram on eukaryotic topoisomerases was studied in the reactions highly specific for topoisomerase I and II. The relaxing activity of topoisomerase I was not affected by thiram at least up to 100 μM concentration (Fig. 1).

In contrast, the unknotting activity of topoisomerase II was clearly inhibited by 10 μM thiram (Fig. 2). Since inhibition of the activities of topoisomerases leads to an increase in the number of recombination events [5], one could speculate that thiram induces SCE [4] *via* its effect on topoisomerase II.

Evaluation of the mutagenic effect of thiram was carried out using the *Salmonella*/mammalian microsome Ames test with *S. typhimurium* TA102. This standard tester strain contains A-T base pairs at the site of the mutation. As it is shown in Table I, thiram was not mutagenic in TA102 (with and without metabolic activation) in the range of nontoxic concentrations. This result excludes cross-links and oxidative damage in DNA as a possible mechanism of mutagenic activity. To check the possibility of thiram's effect on modification of purines we studied the sensitivity of the *fpg*$^-$ mutant to this chemical. The FPG protein of *E. coli* was initially identified as a DNA glycosylase which excises the imidazole ring-opened form of N^7-methylguanine residues in DNA (Fapy) [6]. The FPG protein exhibits a wide substrate specificity, including: the ring-opened form of adenine residues in DNA treated with ionizing radiation, guanine residues modified at the N^7-position with alkylating agents and the ring-opened purines modified at the C^8-position by

[1] Abbreviations: SCE, sister chromatid exchange; FPG protein, the protein encoded by the fapy gene of *E. coli*; MMS, methyl methanesulfonate

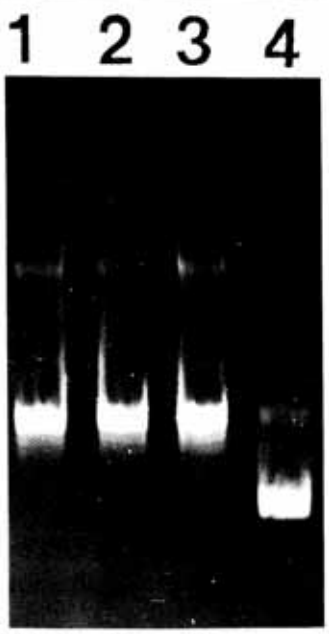

Fig. 1. Effect of thiram on topoisomerase I.
The activity of topoisomerase I was assayed in the nuclear extract from mouse lymphoma L5178Y (LY) cells. Nuclei were isolated according to Pommier *et al.* [7] and extracted with 0.35 M NaCl according to Estley *et al.* [8]. Topoisomerase I assay measured the relaxation of the supercoiled pBR322 by the extract according to Liu [9]. Thiram was mixed with the enzyme in the assay coctail in the absence of the substrate and incubated for 10 min at 30°C. DNA electrophoresis was performed on 0.7% agarose, 2 mM EDTA, 40 mM Tris/acetate buffer, pH 7.8, at 1 V/cm according to Maniatis *et al.* [10]. The concentrations of thiram were: lanes 1, none; 2, 10 µM; 3, 100 µM; 4, control pBR322

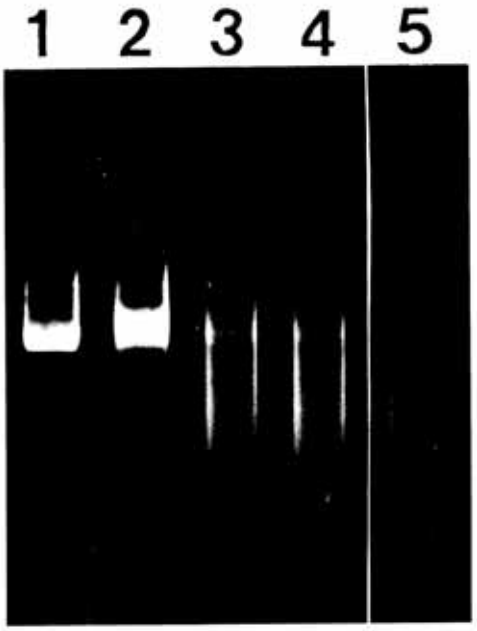

Fig. 2. Effect of thiram on topoisomerase II.
Topoisomerase II assay determined the conversion of topologically knotted phage P4 DNA to the unknotted topoisomer and was performed according to Liu & Davis [11]. Preparation of the nuclear extract, incubation of the extract with thiram and electrophoresis were performed as described in the legend to Fig. 1. The concentrations of thiram were: lanes 1, none; 2, 1 µM; 3, 10 µM; 4, 100 µM; 5, control pBR322

N-hydroksy-2-aminofluoren. The biological importance of this enzyme comes from the fact that Fapy-DNA glycosylase activities have been conserved in prokaryotes and eukaryotes. Figure 3 shows that the sensitivity of bacteria to thiram is not modified in the *fpg*$^-$ strain as compared with the wild type. In both strains a similar survival was observed (e.g. about 70% at 400 µM thiram). This is in contrast with the sensitivity to MMS where significant difference

Table 1

Evaluation of thiram mutagenicity by the Ames test with S. typhimurium strain TA102, and the liver homogenate fraction prepared according to Maron and Ames [12].

Data are means from 12 plates. 50 µl of S9 homogenate fraction was added per plate

Thiram (µg/plate)	number of *his*$^+$ revertants / plate ± S.D.			
	−S9	% ± S.D.	+S9	% ± S.D.
0 + DMSO	460 ±33	100	630 ± 63	100
2	451 ±11	98 ± 2	611 ±130	97 ±21
5	420 ±14	91 ± 3	617 ± 70	98 ±11
10	371 ±59	81 ±13	664 ± 94	105 ±15
25	421 ±55	92 ±12	731 ± 92	116 ±15
50	448 ±50	97 ±11	594 ±123	94 ±20
100	417 ±32	91 ± 7	702 ± 99	112 ±16
150	420 ±20	91 ± 4	625 ± 60	99 ± 9
250	210 ±30	46 ± 7	500 ± 30	79 ± 5

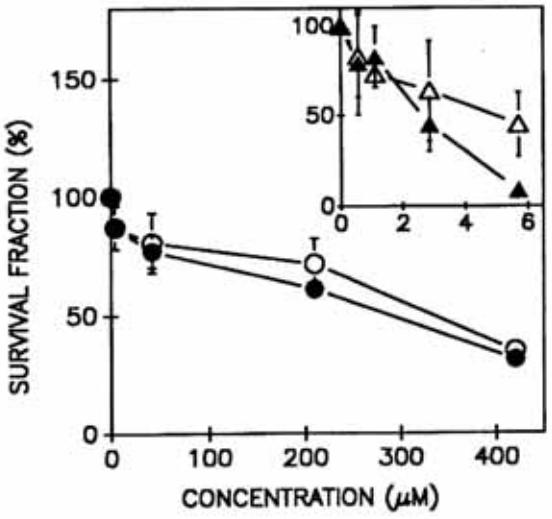

Fig. 3. Effect of thiram and MMS on survival (%) of E. coli AB1157 strain and BH20 fpg⁻-mutant.
The bacteria were grown at 37°C in LB broth supplemented when required with 40 μg/ml kanamycin sulphate. Thiram or MMS was added at the appropriate concentration and the cell suspension was incubated for 60 min at 37°C with agitation. At the end of incubation the suspension was diluted and plated on nutrient agar. The plates were incubated overnight at 37°C. AB 1157 with MMS (Δ) and thiram (○) BH20 with MMS (▲) and thiram (●)

in survival of the mutant and wild *E. coli* strain was revealed (1% and 45%, respectively, at 6 μM MMS).

Such damages, if they did occur, could be excised by UvrABC nucleases [13]. Thus, the results presented imply that the thiram induced lesions do not lead to the opening of the imidazole ring of purines. However this suggestion might be non conclusive since the above mentioned type of damage, could be excised by UvrABC nuclease [13]. It should be noted that the thiram-induced damages in DNA can be removed by the excision repair system in *S. typhimurium* cells [2].

REFERENCES

1. Zdzienicka, M., Zieleńska, M., Tudek, B. & Szymczyk, T. (1979) *Mutat. Res.* **68**, 9 - 13.
2. Zdzienicka, M., Zieleńska, M., Trojanowska, M., Szymczyk, T., Bigham, M. & Carere, A. (1981) *Mutat. Res.* **89**, 1 - 7.
3. Zdzienicka, M., Hryniewicz, M. & Pieńkowska, M. (1982) *Mutat. Res.* **102**, 261 - 264.
4. Pieńkowska, M. & Zieleńska, M. (1990) *Mutat. Res.* **245**, 119 - 123.
5. Wang, J.C., Caron, P.R. & Kim, R.A. (1990) *Cell* **62**, 403 - 406.
6. Boiteux, S., O'Connor, T.R., Lederer, F., Gouyette, A. & Laval, J. (1990) *J. Biol. Chem.* **265**, 3916 - 3922.
7. Pommier, Y., Kerrigan, D., Schwartz, R. & Zwelling, L.A. (1982) *Biochem. Biophys. Res. Commun.* **107**, 576 - 583.
8. Estey, E.H., Silberman, L., Beran, M., Andersson, B.S. & Zwelling, L.A. (1983) *Biochem. Biophys. Res. Commun.* **144**, 787 - 793.
9. Liu, L.F. (1983) *Meth. Enzymol.* **100**, 133 - 137.
10. Maniatis, T., Fritsch, E. & Sambrook, J. (1982) *Molecular Cloning: A Laboratory Manual.* Cold Spring Harbor Laboratories, Cold Spring Harbor, New York.
11. Liu, L.F. & Davis, J.L. (1981) *Nucl. Acids Res.* **9**, 3979 - 3989.
12. Maron, D.H. & Ames, B.N. (1983) *Mutat. Res.* **113**, 173 - 215.
13. Myles, G.M. & Sancar, A. (1989) *Chem. Res. Toxicol.* **2**, 197 - 226.