Note

Antimutagenic Effects of Pyrolysate of Protein on Chemically Induced Mutagenesis in *Escherichia coli*

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Natural products such as vegetable juices and medicinal plants contain factors which inhibit mutagenicity in *Escherichia coli* and *Salmonella* strains (Kada *et al.*).^{1,2)} Various mutagenic compounds have been isolated from cooked foods and the pyrolysates of amino acids and proteins.³⁾ However, among the browning reaction products that are mutagenic, some antimutagenic activity was detected⁴⁾ and cembratriene-4,6-diol was identified from cigarette smoke condensate as an antitumor-agent.⁵⁾ Pyrolysates of protein are complex mixtures and we thought that some factors inhibiting genetic toxicity may be present in pyrolysates of protein. In this paper, the isolation of an antimutagen from the pyrolysate of egg albumin is described.

Sixteen and a half grams of egg albumin were heated in an electric furnace at 500°C for 30 min in air. The anti-

mutagenic effects of the albumin pyrolysate were measured by method of Shimoi *et al.*⁶⁾ *Escherichia coli* B/r WP2 (*trp*⁻) and WP2s (*trp*⁻, *uvrA*⁻) were used as the test strains. Bacteria (WP2 or WP2s) grown overnight in nutrient broth at 37°C were washed twice with phosphate buffer (PB). The bacteria was treated with 4-nitroquinolin 1-oxide (4NQO) or *N*-methyl-*N*-nitro-*N*-nitrosoguanidine (MNNG) at 37°C for 30 min. Treated cells were washed twice with PB and resuspended in PB ($3 \sim 4 \times 10^9$ cells/ml). To 2 ml of a top agar solution, 0.1-ml portions of the treated cell suspension and various amounts of the fractions were added. Each resultant mixture was shaken well and poured onto a semi-enriched minimal (SEM) agar plate. To measure cell viability, the suspension of the treated cells was diluted to 10^6 and a 0.1-ml sample of the diluted suspension of the treated cells was poured onto a plate. *Trp*⁺ revertants and viable cells were counted after incubation at 37°C for 2 days. On MNNG-induced mutagenesis in WP2, a pronounced decrease in the number of *Trp*⁺ revertant colonies was observed on the plate containing albumin pyrolysate (Table I). However, in the case of 4NQO-induced mutagenesis in WP2, albumin pyrolysate slightly enhanced the mutagenicity by 4NQO at doses of up to 2.5 mg per plate, and no antimutagenic effect on 4NQO-induced mutagenesis in WP2s was apparent (data not shown).

To examine whether albumin pyrolysate might inhibit the expression of *Trp*⁺, a reconstruction experiment was done by the method of Shimoi *et al.*⁶⁾ There was scarcely any inhibition of the expression of the *Trp*⁺ phenotype.

For the purification of the active fraction from albumin pyrolysate, the pyrolysate (3.3 g) was dissolved in methanol and chromatographed on a Sephadex LH-20 col-

TABLE I. ANTIMUTAGENIC EFFECTS OF FRACTIONS SEPARATED FROM ALBUMIN PYROLYSATE ON MMMG-INDUCED MUTAGENESIS

	Yields (mg)	Dose (mg/plate)	Mean number of <i>Trp</i> ⁺ colonies/ plate	Viable cells (%)
Albumin pyrolysate	3300	0	2380	100
		1.0	1588	105
		2.5	644	100
		5.0	32	111
		0	1604	100
Fraction 2 (Sephadex LH-20 column)	800	0.5	1424	105
		1.0	1180	101
		2.5	308	95
		0	1720	100
		0.5	1340	103
Sub-fraction 2 (Silica gel column)	324	1.0	1291	103
		2.5	960	93

The bacteria (WP2) was treated with MNNG at 37°C for 30 min. To 2 ml of a top agar solution, 0.1-ml portions of the treated cell suspension and various amounts of fractions were added. Each mixture was shaken well and poured onto a semi-enriched minimal (SEM) agar plate. To measure cell viability, a 0.1-ml sample of the diluted suspension of the treated cells was poured onto a plate. *Trp*⁺ revertants and viable cells were counted after incubation at 37°C for 2 days.

FIG. 1. Fractionation by Chromatography on a Sephadex LH-20 Column.

The albumin pyrolysate dissolved in methanol was chromatographed on a Sephadex LH-20 column using methanol as a eluant and was partitioned into four fractions in accordance with the development of peaks on the yield curve (—●—) and the mutagenic activity curve toward the *Salmonella* strain (TA98).

umn (2.3×10 cm) using methanol as an eluant. As shown in Fig. 1, the pyrolysate was partitioned into four fractions in accordance with the development of both peaks on the yield curve in weight and the mutagenic activity curve toward the *Salmonella* strain. The mutagenic activities of the fractions obtained were assayed by Maron's method.⁷⁾ The bacterial tester strain used was *Salmonella typhimurium* TA98. On MNNG-induced mutagenesis in WP2, fractions 1, 2, and 4 were antimutagenic and fraction 2 was neither toxic nor mutagenic (Table I). Therefore, fraction 2 (0.8 g) was further chromatographed on a silica gel column (2.3×10 cm) stepwise using benzene, 50% ethyl acetate in benzene, ethyl acetate, 10% methanol in ethyl acetate, 20% methanol in ethyl acetate, 50% methanol in ethyl acetate, and methanol as eluants and it was fractionated into seven subfractions. Subfraction 2 obtained from the elution with 50% ethyl acetate in benzene was more active with a dose response up to 5 mg/plate (Table I).

The active subfraction 2 (324 mg) was further purified by chromatography on a silica gel column (1.75×13 cm) stepwise using benzene, 5%, 10%, 15%, and 35% ethyl acetate in benzene as eluants and fractionated into seven portions. Portion 2 obtained from the elution with 5% ethyl acetate in benzene was significantly antimutagenic. Therefore, GC-MS and GC-FT-IR of the portion 2 were done with a Hitachi M-80 and a Nicolet 60SX, respectively. The gas chromatogram obtained from the active portion had two peaks and the mass spectra were in agreement with those of authentic samples of phenol and *p*-cresol, respectively. The GC-FT-IR spectra of the two

FIG. 2. Effects of the Portion 2 (—●—), Phenol (—□—) and *p*-Cresol (—○—) on the MNNG-induced Mutagenesis in WP2.

FIG. 3. Effects of *p*-Cresol on the 4NQO-Induced Mutagenesis in WP2s.

peaks were also similar to those of authentic phenol and *p*-cresol.

Consequently the antimutagenic effects of phenol and *p*-cresol were examined (Fig. 2). *p*-Cresol inhibited mutagenicity initiated by MNNG as well as the active portion 2, but phenol was not antimutagenic. No sample was toxic at doses of up to 5.0 mg per plate. No inhibition of the expression of the Trp⁺ phenotype was observed with *p*-cresol.

It is interesting that the pyrolysate of protein was antimutagenic on MNNG-induced mutagenesis in WP2, although it is mutagenic toward *Salmonella* strains, as already reported.

p-Cresol was identified as the active compound in the antimutagenic portion from the most active fraction of the pyrolysate of albumin. There are other unidentified compounds which are antimutagenic in the pyrolysate of albumin. Direct comparison of antimutagenic activity with mutagenic activity by protein pyrolysates would be difficult, because the detection system for mutagenesis is quite different from that for antimutagenesis. These results indicate the presence of a variety of protective compounds which can modify genetic toxicity in complex mixtures.

With respect to the antimutagenic activity of monophenols, Ohta *et al.* reported on the antimutagenic effect of vanillin and ethylvanillin on 4NQO-induced mutagenesis in WP2s, and stated that it may be due to the enhancement of an error-free recombinational repair system.⁸⁾ To compare the antimutagenic effect of *p*-cresol with those of vanillins, the effects of *p*-cresol on 4NQO-induced mutagenesis in WP2s were measured. *p*-Cresol was weakly active (Fig. 3), but was not toxic to the cells in the concentration used. However, the activity by *p*-cresol on MNNG-induced mutagenesis was higher than that on 4NQO-induced mutagenesis. Therefore, the mechanism of antimutagenesis by *p*-cresol should be different from that by vanillins.

The mechanism of antimutagenesis by *p*-cresol and the antimutagenic effects by phenol derivatives are now being investigated.

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