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Chlorophyllin: a potent antimutagen against environmental and dietary complex mixtures

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Summary

Chlorophyllin, the sodium and copper salt of chlorophyll, was tested for its ability to inhibit the mutagenic activity of a variety of complex mixtures — extracts of fried beef, fried shredded pork, red grape juice, red wine, cigarette smoke, tobacco snuff, chewing tobacco, airborne particles, coal dust and diesel emission particles — in strain TA98 of *Salmonella typhimurium*. Chlorophyllin was highly effective against the mutagenicity (90–100% inhibition) of 8 of these 10 mixtures. The mutagenicity of the other 2 mixtures was inhibited 75–80% at the highest concentration of chlorophyllin studied. Control and reconstruction experiments showed that chlorophyllin was not toxic to Salmonella at the concentrations used. The antimutagenic activity of chlorophyllin was heat-stable. The mechanism of the antimutagenicity of chlorophyllin in these experiments is not known; however, chlorophyllin is an antioxidant. Scavenging of radicals and/or interaction with the active group of mutagenic compounds may be responsible for its antimutagenic activity. The data reported here indicate that chlorophyllin is potentially useful as an antimutagenic agent.

The National Institute for Occupational Safety and Health has estimated that millions of workers in the manufacturing sector have been exposed to potentially hazardous chemicals (NIOSH, 1982), many of which are mutagenic and/or carcinogenic. Exposure of the human population to mutagens and carcinogens is not limited to occupational settings. For example, mutagens are found in airborne particles (Chrisp and Fisher, 1980; Hughes et al., 1980), diesel engine emission (Pepelko et al., 1980), cigarette smoke (DeMarini, 1983), tobacco (Whong et al., 1984, 1985) and beverages and foods (Ames, 1983). Therefore, the potential for frequent human exposure to mutagens and carcinogens exists. Through the effort of regulatory agencies and the scientific community, the level of mutagens and carcinogens may be reduced in our environment and workplaces, but it is unlikely that exposure of humans to these agents can be completely eliminated. The consequence to humans of

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exposure to mutagens is not known in most cases. Nevertheless, damage to DNA by environmental mutagens is likely to be a major cause of cancer and genetic disorders, and may contribute to heart disease, aging and developmental birth defects (Ames, 1979). As there is no known safe concentration or threshold limit, there should be increased efforts to identify agents that can counteract or eliminate the activity of man-made and naturally occurring mutagens in our environment. Such agents, generally referred to as antimutagens, should have a practical use in humans.

Various agents — including vitamins (A, C, and E), glutathione, propylgallate, retinylacetate, germanium oxide, β-carotene, selenium, uric acid, phenol, cinnamaldehyde, cobaltous chloride and coumarin — have been shown to inhibit the mutagenic and/or carcinogenic activity of certain chemicals (see Ames, 1983 and Kada et al., 1985 for refs.).

Nevertheless, some of these agents do not appear to be effective antimutagens and others are toxic at high concentrations. Vitamin C, an extensively studied antimutagen and anticarcinogen, for instance, has been shown to be mutagenic (Shamberger, 1984).

Chlorophyll has been shown to be responsible for most of the antimutagenic activity of certain vegetable extracts (Kimm et al., 1982; Lai, 1979; Lai et al., 1980). Chlorophyllin, the sodium and copper salt of chlorophyll, has been reported to inhibit the mutagenic activity of several known mutagens (Arimoto et al., 1980a; Katoh et al., 1983; Kimm et al., 1982; Lai, 1979; Lai et al., 1980), meat extract (Münzner, 1981), and amino acid pyrolysis products (Arimoto et al., 1980b). Because humans are exposed mainly to environmental and dietary complex mixtures rather than single chemicals, we have studied the antimutagenic activity of chlorophyllin against a variety of such mixtures.

Diesel emission particles, airborne particles, coal dust, tobacco snuff, chewing tobacco, fried shredded pork, fried beef, red grape juice and red wine used in this study were extracted and/or concentrated with the reported procedures (Bjeldanes et al., 1982; Ong et al., 1985; Sousa et al., 1985; Whong et al., 1981, 1984, 1985). Coal dust and

TABLE 1
INHIBITORY EFFECT OF CHLOROPHYLLIN ON THE MUTAGENICITY OF COMPLEX MIXTURES[a] IN *Salmonella typhimurium* TA98

Sample[b] (dose/plate)	Chlorophyllin (mg/plate)								
	0[c]	0	0.16	0.31	0.63	1.25	2.5	5	10
Chlorophyllin[d] control	29	29	—	—	—	26	33	24	21
Diesel emission[e] particles (1 mg)	33	281	—	166	91	58	39	—	—
Airborne particles[e] (4 mg)	33	353	119	71	38	40	—	—	—
Coal dust[e] (62.5 mg)	33	733	—	—	168	141	87	51	—
Cigarette smoke[f] (0.02 cigarette)	36	587	281	142	59	36	—	—	—
Tobacco snuff[e] (85 mg)	25	293	—	—	—	155	113	97	78
Chewing tobacco[e] (100 mg)	25	137	—	—	—	71	67	68	53
Fried shredded[f] pork (3 g)	23	350	234	99	46	25	—	—	—
Fried beef[f] (0,75 g)	23	782	737	485	98	26	—	—	—
Red grape[f] juice (50 ml)	45	239	—	—	—	133	96	72	61
Red wine[f] (50 ml)	45	335	—	—	—	221	160	101	72

[a] At least 3 Expts. were performed for each complex mixture. Two plates were used for each concentration in each experiment. The numbers shown in the Table are average numbers of revertants per plate.

[b] The numbers in parentheses are the amounts of the original materials present in 0.1 ml of extract.

[c] Number of spontaneous revertants per plate.

[d] Tested with and without S9. The numbers shown were without S9. With S9, there were 4–9 additional colonies per plate.

[e] Tested with and without S9. The numbers shown were without S9. Similar reponses were found with S9.

[f] Tested with S9. S9 was required for the mutagenic activity or for a greater mutagenic response.

chewing tobacco extracts were nitrosated by sodium nitrite. For the cigarette smoke, smoke from a burning cigarette was trapped in acetone. After evaporation of the acetone, the extract was redissolved in dimethyl sulfoxide. Various amounts of these extracts were tested for mutagenic activity in the Salmonella/mammalian microsome test using strain TA98 of *Salmonella typhimurium* with or without mammalian S9 mix (Ames et al., 1975). After 2 days of incubation at 37°C, the number of revertants per plate was scored. A mutagenic concentration of each extract (producing approximately 200–700 colonies/plate) was selected for the experiments using chlorophyllin. Various concentrations of chlorophyllin (Sigma Chemical Co., St. Louis, MO) were tested with one concentration of the extract of each complex mixture, again using strain TA98. An overnight culture of TA98 (0.1 ml), a test sample (0.1 ml), and chlorophyllin (0.1 ml of a distilled H_2O solution) with (0.5 ml) or without S9 mix were added to 2 ml of molten top agar. The S9 used was prepared from the liver of Aroclor-1254 (500 mg/kg body weight) pretreated male Wistar rats. The mixture was overlayed on a Vogel-Bonner agar plate. After 2 days of incubation, the numbers of revertants per plate were scored and percents of inhibition of mutagenic activity by chlorophyllin were determined.

The results are presented in Table 1 and Fig. 1. Chlorophyllin alone was neither mutagenic nor toxic. Chlorophyllin inhibited the mutagenic activity of each of the complex mixtures in a dose-dependent manner. It was a very effective inhibitor of the mutagenic activity of extracts of airborne particles, cigarette smoke, fried shredded pork and fried beef. Generally, complete inhibition was obtained with 1.25 mg of chlorophyllin per plate. It also inhibited strongly (92–98%) the mutagenic activity of extracts of diesel emission particles, coal dust, red grape juice and red wine, but at concentrations of chlorophyllin greater than 1.25 mg per plate. The only complex mixtures whose mutagenicities were inhibited less than 90% by chlorophyllin were extracts of tobacco snuff and chewing tobacco, which were inhibited 75–80% by 10 mg of chlorophyllin per plate.

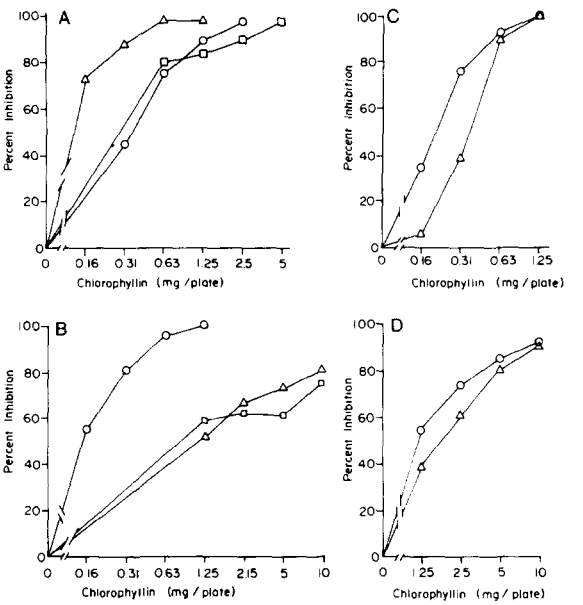

Fig. 1. Percent inhibition (PI) was calculated by the following formula:

$$PI = 100 - \frac{\text{Number of revertants per plate in the presence of chlorophyllin}}{\text{Number of revertants per plate in the absence of chlorophyllin}} \times 100$$

The number of spontaneous revertants per plate was subtracted from the numerator and denominator. (A) Diesel emission particles, ○; airborne particles, △; coal dust, □. (B) Cigarette smoke, ○; tobacco snuff, △; chewing tobacco, □. (C) Fried shredded pork, ○; fried beef, △. (D) Red grape juice, ○; red wine, △.

The determine whether the antimutagenic property of chlorophyllin is heat-resistant, a sample of chlorophyllin was heated for 10 min at 100°C. The antimutagenic activities of the heated and unheated chlorophyllin were similar when tested with diesel emission particles. Controls of chlorophyllin alone (line one in Table 1) indicated that chlorophyllin was not toxic to Salmonella at the concentrations used. There was no obvious decrease in the number of spontaneous revertants of TA98, and there was no inhibition of the background growth of TA98 on the plates. We used reconstruction experiments to further determine whether chlorophyllin inhibited the growth of revertants of TA98. 15 Revertants colonies induced by fried beef extract were picked from the

plate and mixed. Then, 100, 250, 800 or 1200 revertant cells from this mixture were plated along with the usual 0.1 ml of an overnight culture of TA98 in the absence or presence of 5 mg of chlorophyllin per plate. The revertant colonies were counted after 2 days of incubation. The results showed that the recovery of revertants was approximately 100% at the different numbers of revertants plated in the absence or presence of chlorophyllin. These results clearly indicate that the decreased number of revertants observed in the previous experiments was due to inhibition of the mutagenic activity of the complex mixture extracts and not to inhibition of cell growth by chlorophyllin.

The inhibitory action of chlorophyllin encompassed a wide variety of chemical compounds such as nitropyrenes (airborne and diesel emission particles), nitroso compounds (coal dust, tobacco snuff and chewing tobacco), flavonoids (red wine and grape juice), aromatic amines (cigarette smoke), and other polycyclic hydrocarbons (cigarette smoke, airborne particles, diesel emission particles, fried beef and fried shredded pork). The mechanism by which chlorophyllin inhibits mutagenic activity is not known. It is known to be an antioxidant (Sato et al., 1977, 1984). The antioxidation by chlorophyllin can be either enzymatic or nonenzymatic. Chlorophyllin a has been reported to be an effective photoreducing agent (Brune and Pietro, 1970). Therefore, chlorophyllin may act as an antimutagen by scavenging radicals and/or interacting with the active groups of mutagenic compounds.

Chlorophyllin has been used to control body, fecal and urinary odor of geriatric patients (Nahata et al., 1983; Young and Beregi, 1980). It may be useful for the acceleration of wound healing (Krasnikova, 1973) and for the treatment of calcium oxalate stone disease (Berg et al., 1982; Tawashi et al., 1982). No significant toxic effect of chlorophyllin has been reported in human and animal species. Further studies on the antimutagenic and anticarcinogenic activities, and the possible toxicity, of chlorophyllin are needed. If no toxicity is associated with chlorophyllin and if it possesses anticarcinogenic activity, the addition of chlorophyllin to certain beverages, foods, chewing tobacco and tobacco snuff should be considered. Increased use of chlorophyllin in the diet may be a simple and effective way to reduce the potential health hazards of mutagens and/or carcinogens in our environment, especially in certain occupational settings.

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