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Hemolytic Anemia and Induction of Phase II Detoxification Enzymes by Diprop-1-enyl Sulfide in Rats: Dose−Response Study

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Epidemiological evidence indicates that a high dietary intake of plants of the *Allium* family, such as garlic and onions, is associated with a decreased risk of cancer in humans. It has been suggested that this chemopreventative effect involves the ability of the aliphatic sulfides derived from these vegetables to increase tissue activities of phase II detoxification enzymes. Several highly effective inducers from garlic have been identified, but most of the previously studied compounds from onion have proved to be only weakly active. In the present study, the inductive activity of another onion-derived sulfide, diprop-1-enyl sulfide, has been investigated. This substance was a potent inducer of phase II enzymes in rats, showing significant effects in the lungs and in the lower part of the gastrointestinal tract, suggesting that diprop-1-enyl sulfide could be a useful chemopreventative agent at these sites. At high dose levels, diprop-1-enyl sulfide caused hemolytic anemia, which may be due to in vivo conversion of the sulfide to active metabolites.

KEYWORDS: ***Allium*** **vegetables; chemoprevention; diprop-1-enyl sulfide; phase II enzyme induction; quinone reductase; glutathione *S*-transferase; hemolysis**

INTRODUCTION

Plants of the *Allium* family, such as onions, garlic, leeks, shallots, and chives, have been cultivated for food since earliest times (*1*). They are particularly valued for their characteristic organoleptic properties, which derive from the presence of alk(en)yl cysteine sulfoxides within the plant tissue (*2*). When the tissue is broken by cutting or chewing, the cysteine derivatives are enzymatically degraded to thiosulfinates. The latter compounds spontaneously decompose to a complex mixture of compounds, in which monosulfides, disulfides, trisulfides, and tetrasulfides predominate (*3*); these same compounds are produced through in vivo metabolism of thiosulfinates in humans and animals (*4*−*6*).

Propyl, allyl, and prop-1-enyl cysteine sulfoxides are present in high concentration in Alliaceae. On decomposition, these compounds may yield symmetrical sulfides, in which the two alk(en)yl groups are the same, or mixed sulfides, in which they are different. Garlic contains mainly allyl cysteine sulfoxide, and the major sulfide breakdown products are therefore allyl mono-, di-, tri-, and tetrasulfide. In contrast, propyl and prop-1-enyl cysteine sulfoxide predominate in onions (*2*, *7*), and these yield symmetrical and mixed propyl and prop-1-enyl sulfides (*8*−*10*).

Hemolytic anemia has been observed in domestic and farm animals after consumption of plants of the *Allium* family, which is attributable to the damage induced in erythrocytes by the thiosulfinate breakdown products, leading to their sequestration and destruction in the spleen (*11*). In rats, diprop-1-enyl disulfide is a potent hemolytic agent, whereas the diallyl and dipropyl disulfides are much less active (*12*). Tri- and tetrasulfides are strongly hemolytic, with higher activity than disulfides (*13*). The hemolysis induced by the *Allium*-derived sulfides is of the oxidative type, as indicated by the presence of irreversibly oxidized hemoglobin, recognized as Heinz bodies, within erythrocytes. The oxidative damage to red blood cells results from the intracellular generation of "active oxygen" species through redox cycling. Reduction of the sulfur−sulfur bond in disulfides or polysulfides forms the corresponding thiol or perthiol, which undergoes oxidation, catalyzed by oxyhemoglobin, with consequent formation of hydrogen peroxide (*13*). Dipropyl sulfide and diallyl sulfide do not cause hemolysis in rats even at high dose levels (*13*), consistent with the fact that, lacking a sulfur−sulfur bond, monosulfides cannot undergo redox cycling in the same way as the higher sulfides.

No adverse effects from human consumption of *Allium* vegetables have been reported. Indeed, epidemiological studies suggest that such consumption may be beneficial, with individuals consuming large amounts of garlic or onions showing a decreased incidence of cancer, particularly cancer of the stomach and intestine (*14*). This effect may again be attributable to the

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alk(en)yl sulfides, because several such compounds have been shown to protect animals against chemical carcinogens (*15*, *16*). There is evidence that this effect is due, at least in part, to the ability of the sulfides to increase tissue activities of phase II detoxification enzymes (*17*, *18*). These enzymes, which include glutathione *S*-transferase (GST, EC 2.5.1.18) and NAD(P)H: quinone acceptor oxidoreductase (QR, DT-diaphorase, EC 1.6.99.2), inactivate carcinogens and facilitate their excretion from the body (*19*, *20*).

Among the compounds derived from garlic, diallyl di-, tri-, and tetrasulfide are potent inducers of phase II enzymes in rats (*13*, *21*), suggesting that these substances may contribute to the observed chemoprotective action of this vegetable. In contrast, all of the propyl sulfides derived from onions are weak inducers, and diprop-1-enyl disulfide is of only moderate activity (*22*), indicating that these substances are unlikely to be involved in the anticancer action of onions. The most active onion-derived sulfide that has been identified so far is diprop-1-enyl sulfide, although in previous studies with this substance (*22*), only a single, high, dose level was employed.

To provide more information on the inductive potency of diprop-1-enyl sulfide, a dose−response experiment on phase II enzyme induction in rat tissues has been conducted. In this experiment, evidence of a hemolytic effect of this substance was unexpectedly encountered. This effect was further investigated by comparison of the hematological effects of diprop-1-enyl sulfide with those of diprop-1-enyl disulfide.

MATERIALS AND METHODS

Chemicals. Diprop-1-enyl sulfide was synthesized according to the method of Trofimov et al. (*23*), which yields a mixture of the *Z*,*Z*, *E*,*Z*, and *E*,*E* isomers in the ratio of 1.5:2:1. Diprop-1-enyl disulfide was prepared according to the method of Wijers et al. (*24*). Reagents for enzyme assay were purchased from Sigma.

Animals and Housing. Female Sprague−Dawley rats (10−11 weeks old) from the Ruakura colony were randomly allocated to treatment groups. The animals were housed in solid-bottom cages containing bedding of wood shavings and were allowed free access to food (Laboratory Chow, Sharpes Animal Feeds, Carterton, New Zealand) and water throughout the experiment. Room temperature was maintained at 21−23 °C with a 12 h light/dark cycle.

Dosing and Necropsy. In the experiment on phase II enzyme induction, groups of five rats were given diprop-1-enyl sulfide by oral intubation at dose levels of 10, 25, 50, 100, 200, and 500 μmol/kg/day for 5 days. The test material was administered as a freshly prepared solution in soybean oil, with the volume of vehicle being kept constant at 2 mL/kg. Ten control rats were dosed with soybean oil alone. On the sixth day of the experiment, the rats were anesthetized with halothane and killed by exsanguination, blood being taken into EDTA-containing tubes from the posterior vena cava. The liver, kidney, and spleen of each animal were weighed, and a portion of the liver, together with the kidneys, spleen, heart, urinary bladder, and lungs were placed in plastic vials. The gastrointestinal tract was separated into forestomach, glandular stomach, duodenum, jejunum, ileum, cecum, and colon plus rectum. These tissues were cut lengthways, and the contents were washed out with cold running water. They were then gently blotted on absorbent paper and, along with the other organs, stored at −80 °C before analysis.

In the experiment on the hematological effects of diprop-1-enyl sulfide and disulfide, the test compounds were administered to rats as solutions in soybean oil at a dose of 500 μmol/kg/day for 5 days. Five control rats received vehicle alone. On the sixth day, the rats were killed as described above, and the liver, kidneys, and spleen of each animal were excised and weighed; samples were preserved in 4% buffered formaldehyde. Paraffin wax sections of fixed tissues were stained with hematoxylin and eosin for examination by light microscopy. Sections were examined without reference to treatment group. Splenic and hepatic erythropoietic activities were scored on an arbitrary scale of 0−5, 0 indicating the absence of activity and 5 its presence to a marked degree. The packed cell volume of each blood sample was determined by the microhematocrit technique, whereas hemoglobin levels were assessed according to the cyanmethemoglobin method (*25*). Blood films were stained with New Methylene Blue for reticulocyte and Heinz body counts (*26*).

Table 1. Blood Packed Cell Volumes, Hemoglobin Levels, and Relative Splenic Weights of Rats Receiving Diprop-1-enyl Sulfide at Various Dose Levels[a]

dose (μmol/kg/day)	blood packed cell volume (%)	blood hemoglobin level (g/100 mL)	rel splenic wt (g/100 g of body wt)
0 (control)	45.7 ± 0.4	148 ± 5	0.195 ± 0.012
10	44.9 ± 0.8	146 ± 3	0.210 ± 0.011
25	44.8 ± 0.6	144 ± 4	0.200 ± 0.010
50	44.4 ± 0.9	140 ± 2	0.189 ± 0.011
100	44.3 ± 0.4	142 ± 3	0.183 ± 0.010
200	42.1 ± 0.6*	137 ± 2*	0.205 ± 0.012
500	39.2 ± 0.3***	124 ± 2***	0.259 ± 0.013**

[a] Values shown are the means ± SEM of the 5 or 10 animals per group. Entries marked with asterisks are significantly different from the control: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Enzyme Assays. Tissues were homogenized and centrifuged as described previously (*15*). QR was assayed at 25 °C according to the method of Ernster (*27*) and for GST according to the method of Habig et al. (*28*), using 1-chloro-2,4-dinitrobenzene as substrate. Enzyme activities were calculated as International Units (IU) per gram of tissue. Statistical significance of the data was tested by two-way analysis of variance followed by Student's *t* test, using InStat software (GraphPad, San Diego, CA).

RESULTS

Rats receiving diprop-1-enyl sulfide remained in good health throughout the experimental period, and their body weights showed no significant difference from those of control animals (data not shown). At necropsy, darkening of the spleens of rats given 500 μmol/kg/day of the test compound was noted, and the spleens of these animals were significantly heavier than those of controls (**Table 1**). No effects on renal or hepatic weights were observed (data not shown). The blood packed cell volumes and hemoglobin levels of rats dosed with diprop-1-enyl sulfide at 200 and 500 μmol/kg/day were decreased (**Table 1**).

At the two highest dose levels, diprop-1-enyl sulfide increased QR activities in all 13 tissues analyzed and increased GST in all tissues except the heart (**Tables 2**−**5**). Significantly increased QR and/or GST activities were recorded in the kidneys and jejunum of the animals at 50−200 μmol/kg/day and in the urinary bladder, glandular stomach, and forestomach at 25−200 μmol/kg/day of the test compound, with the greatest effects in these organs being seen with QR. The tissues most susceptible to the inductive effect of diprop-1-enyl sulfide were the lungs, duodenum, ileum, cecum, and colon plus rectum, in which significant increases in QR and/or GST activity were recorded at a dose of only 10 μmol/kg/day.

The increased splenic weights and decreased packed cell volumes observed in rats receiving high dose levels of diprop-1-enyl sulfide were confirmed in a second experiment, although the severity of these changes was less that that seen in animals receiving an equivalent dose of diprop-1-enyl disulfide (**Table 6**). Heinz bodies were present in the erythrocytes of animals dosed with both compounds, and splenic erythropoietic activity was increased. Increased erythropoietic activity in the liver was recorded in animals receiving diprop-1-enyl disulfide, and levels of circulating reticulocytes were increased, but no changes in

Table 2. QR Activities in the Spleen, Liver, Kidneys, Heart, Lungs, and Urinary Bladder of Rats Receiving Diprop-1-enyl Sulfide at Various Dose Levels[a]

dose (μmol/kg/day)	activity (IU/g)					
	spleen	liver	kidneys	heart	lungs	urinary bladder
0 (control)	0.66 ± 0.09	34.2 ± 2.5	2.98 ± 0.28	0.96 ± 0.09	11.6 ± 0.4	13.9 ± 1.5
10	0.75 ± 0.20	37.3 ± 3.1	3.36 ± 0.37	0.72 ± 0.07	14.9 ± 1.0*	17.3 ± 0.6
25	1.05 ± 0.22	35.9 ± 2.5	3.85 ± 0.42	1.03 ± 0.14	17.5 ± 1.1***	18.9 ± 1.2*
50	1.44 ± 0.32	45.7 ± 2.3	4.86 ± 0.44**	0.90 ± 0.07	19.6 ± 1.5***	21.3 ± 2.6*
100	1.42 ± 0.26	39.6 ± 2.8	4.72 ± 0.33**	1.05 ± 0.07	21.5 ± 2.0***	26.2 ± 1.0***
200	2.09 ± 0.28**	58.6 ± 8.6**	7.65 ± 0.54***	1.29 ± 0.19*	29.0 ± 1.6***	39.0 ± 3.9***
500	2.15 ± 0.28**	58.7 ± 6.4**	11.09 ± 0.93***	1.21 ± 0.12*	30.9 ± 2.9***	40.4 ± 2.4***

[a] Values shown are the means ± SEM of the 5 or 10 animals per group. Entries marked with asterisks are significantly different from the control: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 3. QR Activities in the Glandular Stomach, Forestomach, Duodenum, Jejunum, Ileum, Cecum, and Colon plus Rectum of Rats Receiving Diprop-1-enyl Sulfide at Various Dose Levels[a]

dose (μmol/kg/day)	activity (IU/g)						
	glandular stomach	forestomach	duodenum	jejunum	ileum	cecum	colon plus rectum
0 (control)	39.0 ± 2.4	9.8 ± 0.7	6.1 ± 0.5	2.62 ± 0.17	11.3 ± 1.4	41.9 ± 2.3	23.0 ± 1.5
10	42.9 ± 1.6	11.4 ± 0.9	7.7 ± 0.4*	3.24 ± 0.27	12.9 ± 1.0	53.8 ± 1.3***	27.5 ± 1.1*
25	46.9 ± 2.1*	13.1 ± 1.5*	10.0 ± 0.7***	3.55 ± 0.42	14.5 ± 1.1***	55.3 ± 1.7***	29.1 ± 1.1**
50	54.2 ± 1.6***	16.7 ± 1.0***	16.1 ± 0.7***	5.69 ± 0.61***	19.1 ± 1.5***	71.0 ± 3.6***	36.0 ± 1.7***
100	59.7 ± 3.7***	22.3 ± 0.7***	18.1 ± 1.7***	6.42 ± 0.88***	20.3 ± 2.0***	74.0 ± 4.9***	37.0 ± 3.2***
200	81.1 ± 4.5***	34.2 ± 0.5***	33.2 ± 2.8***	12.70 ± 1.05***	29.2 ± 0.7***	125.8 ± 3.9***	60.4 ± 2.4***
500	100.1 ± 3.7***	50.9 ± 2.8***	43.1 ± 1.3***	21.01 ± 1.34***	34.7 ± 2.9***	173.6 ± 7.4***	71.3 ± 3.2***

[a] Values shown are the means ± SEM of the 5 or 10 animals per group. Entries marked with asterisks are significantly different from the control: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 4. GST Activities in the Spleen, Liver, Kidneys, Heart, Lungs, and Urinary Bladder of Rats Receiving Diprop-1-enyl Sulfide at Various Dose Levels[a]

dose (μmol/kg/day)	activity (IU/g)					
	spleen	liver	kidneys	heart	lungs	urinary bladder
0 (control)	5.57 ± 0.12	113 ± 3	14.5 ± 0.4	3.01 ± 0.09	5.92 ± 0.29	3.21 ± 0.13
10	5.55 ± 0.18	123 ± 5	14.4 ± 0.5	3.32 ± 0.48	6.66 ± 0.35*	2.76 ± 0.10
25	5.53 ± 0.16	126 ± 6	14.5 ± 0.4	3.41 ± 0.06	7.18 ± 0.24**	3.04 ± 0.12
50	5.91 ± 0.10	123 ± 3	15.8 ± 0.7	3.14 ± 0.13	7.57 ± 0.28***	3.21 ± 0.15
100	6.60 ± 0.15**	127 ± 6	16.4 ± 0.7	3.05 ± 0.05	8.17 ± 0.20***	3.39 ± 0.18
200	6.47 ± 0.23**	152 ± 10***	17.5 ± 0.9*	3.32 ± 0.15	8.88 ± 0.35***	4.43 ± 0.46**
500	7.06 ± 0.33***	175 ± 7***	23.4 ± 0.9***	3.21 ± 0.10	9.91 ± 0.34***	5.48 ± 0.52***

[a] Values shown are the means ± SEM of the 5 or 10 animals per group. Entries marked with asterisks are significantly different from the control: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Table 5. GST Activities in the Glandular Stomach, Forestomach, Duodenum, Jejunum, Ileum, Cecum, and Colon plus Rectum of Rats Receiving Diprop-1-enyl Sulfide at Various Dose Levels[a]

dose (μmol/kg/day)	activity (IU/g)						
	glandular stomach	forestomach	duodenum	jejunum	ileum	cecum	colon plus rectum
0 (control)	7.03 ± 0.38	2.67 ± 0.12	14.1 ± 2.0	12.5 ± 0.8	4.32 ± 0.17	5.56 ± 0.12	2.74 ± 0.09
10	8.15 ± 0.21	3.03 ± 0.14	20.7 ± 1.5**	13.9 ± 0.9	5.09 ± 0.24*	6.08 ± 0.20	2.70 ± 0.22
25	8.00 ± 0.45	3.05 ± 0.19	22.9 ± 1.9***	14.4 ± 0.6	5.04 ± 0.09*	6.03 ± 0.16	3.04 ± 0.09
50	8.44 ± 0.39	3.33 ± 0.19	25.2 ± 1.3***	15.7 ± 1.1	5.14 ± 0.12*	6.14 ± 0.31	3.12 ± 0.10
100	10.58 ± 1.12***	3.54 ± 0.17*	29.8 ± 1.9***	18.1 ± 0.8**	5.66 ± 0.29***	6.33 ± 0.24	3.29 ± 0.13*
200	12.50 ± 0.60***	4.65 ± 0.18***	35.3 ± 2.1***	25.5 ± 1.9***	6.17 ± 0.15***	8.08 ± 0.16***	3.52 ± 0.12**
500	16.05 ± 0.85***	6.33 ± 0.57***	38.3 ± 2.5***	28.1 ± 1.9***	7.70 ± 0.41***	10.89 ± 0.49***	5.18 ± 0.24***

[a] Values shown are the means ± SEM of the 5 or 10 animals per group. Entries marked with asterisks are significantly different from the control: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

these parameters were observed in rats dosed with diprop-1-enyl sulfide (**Table 6**).

DISCUSSION

In accord with previous experiments (*22*), diprop-1-enyl sulfide was a potent inducer of QR and GST in rats, with an activity comparable to that of diallyl disulfide, one of the most powerful enzyme inducers derived from *Allium* vegetables so far identified. The organ specificity of induction by diprop-1-enyl sulfide was, however, quite different from that of diallyl disulfide. The latter compound is most effective in the stomach, duodenum, and jejunum of rats, with comparatively little effect

Table 6. Hematology, Splenic Weights, and Splenic and Hepatic Histology of Rats Receiving Diprop-1-enyl Sulfide or Diprop-1-enyl Disulfide at a Dose of 500 μg/kg/Day[a]

compound	blood packed cell volume (%)	blood hemoglobin level (g/L)	Heinz body count[b]	reticulocyte count[c]	rel splenic wt (g/100 g of body wt)	splenic erythropoietic activity[d]	hepatic erythropoietic activity[d]
none (control)	42.8 ± 0.4	137 ± 2	0.0	1.5 ± 0.2	0.245 ± 0.004	0.25 ± 0.17	0.17 ± 0.11
diprop-1-enyl sulfide	39.8 ± 0.6**	126 ± 3**	10.3 ± 0.4*	1.3 ± 0.4	0.303 ± 0.017**	1.83 ± 0.33**	0.17 ± 0.11
diprop-1-enyl disulfide	33.3 ± 0.7***	100 ± 2***	31.5 ± 5.8***	7.6 ± 2.0***	0.446 ± 0.022***	3.42 ± 0.33***	0.58 ± 0.08**

[a] Values shown are the means ± SEM of the five animals per group. Entries marked with asterisks are significantly different from the control: *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. [b] Percentage of cells in blood film containing one or more Heinz bodies. [c] Percentage of reticulocytes in blood film. [d] Scored on an arbitrary scale of 0–5, as described under Materials and Methods.

being observed in the lower part of the gastrointestinal tract (*21*). In contrast, diprop-1-enyl sulfide was most effective in the ileum, cecum, and colon plus rectum. Furthermore, diprop-1-enyl sulfide showed a greater effect than diallyl disulfide in the kidneys, lungs, and urinary bladder, with significant effects being recorded at much lower dose levels than those required for induction by the disulfide.

In the case of diallyl disulfide, the dose levels needed to increase phase II enzyme activity in the stomach and upper intestine in rats are similar, on a milligram per kilogram of body weight basis, to those that could be achieved by humans through consumption of a diet containing a high level of garlic (*21*). Diallyl disulfide, along with the trisulfide and tetrasulfide, which are also potent phase II enzyme inducers (*13*), are major components of processed garlic (*8*), and it was suggested (*21*) that the effects of these substances on phase II enzyme activity could account, at least in part, for the chemoprotective effect of this vegetable. The question then arises as to whether diprop-1-enyl sulfide could similarly contribute to the observed anticancer effect of onions.

The presence of diprop-1-enyl sulfide has been reported in various onion preparations (*10*, *29*, *30*). No detailed quantitative data are available, although it would appear that the concentration of all prop-1-enyl sulfides is small in proportion to the corresponding propyl sulfides, due to the multiplicity of decomposition products that are formed from prop-1-ene thiosulfinate (*10*). In the present experiment, significant increases in phase II enzyme activity were seen in rats given diprop-1-enyl sulfide at 10 μmol/kg/day, which is equivalent to 1.14 mg/kg/day. Such a dose, however, would correspond to an intake of 68 mg/day for a 60 kg human. The average intake of onions in the United Kingdom and the United States is ~14 g/day, and onions contain prop-1-enyl cysteine sulfoxide at ~2−3 mg/g of fresh weight (*31*−*33*). To achieve the above intake, therefore, even in individuals with an onion intake 5 times the average, quantitative conversion of the prop-1-enyl cysteine sulfoxide to the sulfide would be necessary. This is patently not the case (*10*), and it is unlikely that diprop-1-enyl sulfide, by way of its effect on phase II enzyme activity, plays a major role in the chemopreventative effect of onions. Studies on other breakdown products of prop-1-enyl cysteine sulfoxide, such as cepaenes, zweibelanes and thiophene derivatives (*10*), would be of interest.

The hematological and splenic weight changes, indicative of hemolysis, in animals receiving high doses of diprop-1-enyl sulfide were quite unexpected, because this substance, being a monosulfide, cannot generate redox-active thiols or perthiols through cleavage of a sulfur−sulfur bond. The hemolytic effect of diprop-1-enyl sulfide was investigated in more detail in a comparative study with diprop-1-enyl disulfide, which is known to be a powerful hemolytic agent in rats (*12*). The latter substance redox cycles between the disulfide and the corresponding thiol, prop-1-enethiol, with concomitant generation of oxidizing species that irreversibly damage erythrocytes. The hemolytic effect of diprop-1-enyl disulfide was confirmed in the present experiment. This was characterized by anemia, Heinz body formation, reticulocytosis, splenic enlargement, and histological evidence of compensatory erythropoiesis in both the spleen and the liver. In the absence of a disulfide bond, such redox cycling cannot occur with diprop-1-ene sulfide, yet this substance, like the disulfide, caused oxidative hemolysis. The severity of the effect was lower than that of an equivalent dose of the disulfide, but anemia, Heinz body formation, splenic enlargement, and increased erythropoiesis were seen in rats receiving diprop-1-enyl sulfide. It is possible that this effect is due to in vivo metabolism of diprop-1-enyl sulfide. Although the metabolism of this substance in animals has not been investigated, various monosulfides have been shown to undergo dealkylation by a microsomal enzyme (*34*), and diallyl sulfide is known to be dealkylated in rats (*35*). In the case of diprop-1-enyl sulfide, dealkylation would generate prop-1-enethiol, the same thiol that is formed by reduction of diprop-1-enyl disulfide, which could then redox cycle to cause the observed oxidative damage to erythrocytes.

Many different classes of chemical have been shown to induce phase II enzymes, and most are known to be chemically reactive electrophiles that modify thiol groups by alkylation, oxidation, or reduction (*36*). Such thiol group reactivity leads to transcriptional activation of phase II enzyme genes, which is primarily mediated by the antioxidant response element or the electrophile response element, located on the upstream region of the genes (*37*, *38*). Diprop-1-enyl sulfide, however, is a very stable compound, and direct reaction with tissue thiols seems to be unlikely. Although formation of prop-1-enethiol through dealkylation would certainly generate species capable of oxidizing thiols, this cannot be the explanation for the observed inductive activity because diprop-1-enyl disulfide, which readily generates prop-1-enethiol, is a much weaker phase II enzyme inducer than diprop-1-enyl sulfide (*22*). It is possible, however, that the electrophilic alkyl radical that is also generated during dealkylation could alkylate thiols, thus triggering gene activation. Further work on the mechanism of phase II enzyme induction by diprop-1-enyl sulfide is required. The question also arises as to whether two prop-1-enyl groups are essential for inductive activity or whether mixed sulfides such as prop-1-enyl propyl sulfide would also be active. The symmetrical sulfide would be more delocalized than the mixed sulfide, which could have a major impact on biological activity. It has been shown that the inductive activity of symmetrical methyl, propyl, and allyl thiosulfinates in cultured cells in vitro is 3 orders of magnitude less than that of mixed prop-1-enyl thiosulfinates (*40*, *41*), suggesting that a single prop-1-enyl moiety may be sufficient, but in vivo studies on mixed prop-1-enyl sulfides are needed

to resolve this point. Furthermore, the double-bond stereochemistry in diprop-1-enyl sulfides could also influence inductive activity. Because the diprop-1-enyl sulfide employed in the present experiment was a mixture of stereoisomers, no comment can be made on this possibility, and experiments with pure stereoisomers of diprop-1-enyl sulfide are required.

Although diprop-1-enyl sulfide may not be involved in the chemopreventive action of onions, it remains a very strong inducer of phase II enzymes and may therefore be valuable as a chemopreventative agent in its own right. Unlike other vegetable-derived inducers (*22*, *39*), diprop-1-enyl sulfide showed high inductive activity in the lower gastrointestinal tract and in the lung, and studies on the effect of this substance on tumorigenesis at these sites would be of interest.

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