REVIEWS

Improvement in the Safety of Foods by SH-Containing Amino Acids and Peptides. A Review

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Most food toxicants have specific groups responsible for their deleterious effects. Modifying such sites with site-specific amino acids, peptides, and proteins should lessen their toxicity. Sulfhydryl (SH) groups are particularly suited to achieve this objective because of their great reactivity. The chemical reactivities of SH compounds are much greater than would be expected from their pK values. This enhanced reactivity results from (a) polarization of outer shell sulfur electrons; (b) the availability of d-orbitals in the electronic structure of sulfur, permitting d-orbital overlap during the formation of transition states; and (c) the ability of sulfur to act as a free-radical trap, whereby free electrons in highly reactive oxygen radicals are transferred or dissipated to sulfur atoms. This overview covers the biological utilization and safety of sulfur amino acids and possible approaches to ameliorating adverse effects of representative food ingredients, based on the reactivity of the sulfhydryl group with electrophilic centers. The latter include (a) the double bond of the furan ring of aflatoxins to suppress mutagenicity, (b) the double bond of dehydroalanine to prevent lysinoalanine formation, (c) the conjugated system of quinones to inhibit nonenzymatic and enzymatic browning in potatoes and other foods, and (d) the disulfide bonds of soybean inhibitors of digestive enzymes to facilitate inactivation through sulfhydryl–disulfide interchange. Possible benefits of these transformations to food safety, food quality, nutrition, and health and suggestions for future research are discussed.

INTRODUCTION

Naturally occurring antinutrients as well as food toxicants and those formed during food processing adversely effect the nutritional quality and safety of foods. Because of a growing awareness about direct relationships between diet and diseases (Ames, 1983; Friedman, 1975, 1978b; 1984a, 1986, 1991a,b, 1992a,b; Newberne, 1993; Rosenkrantz and Klopman, 1990), and because of an imperative need to improve the quality and safety of our food supply, more research is needed to define conditions that inhibit or minimize or counteract the formation of nutritionally antagonistic and toxic compounds in foods.

Most naturally occurring food toxicants and many antinutrients possess specific sites that are responsible for their deleterious effects. Therefore, by modifying such sites with site-specific reagents, such as thiols or other compounds, in a manner that will alter structural integrity and thus prevent them from interacting with receptor sites *in vivo*, it should be possible to lessen their toxic potential.

Sulfhydryl groups in amino acids, peptides, and proteins participate in anionic, cationic, and free-radical reactions both *in vitro* and *in vivo* (Friedman, 1973). The chemical reactivities of negatively charged sulfur anions (RS^-) are much greater than would be expected from their basicities. This great reactivity presumably results from (a) polarizabilities of outer shell sulfur electrons and (b) the availability of d-orbitals in the electronic structure of sulfur, permitting d-orbital overlap during the formation of transition states. Besides acting as a precursor for disulfide bonds that stabilize proteins, sulfhydryl groups participate directly in many and varied chemical and biochemical processes. The antioxidant and antitoxic effects of SH-containing amino acids, peptides, and proteins are therefore due to their abilities to act as reducing agents, scavengers of oxygen radicals, precursors of cellular glutathione, and inducers of cellular detoxification. The low bond energy of the disulfide bond (60 kcal/mol) compared to that of the carbon–carbon bond (105 kcal/mol) also facilitates some of the chemical and biochemical transformations described below.

This limited review uses examples largely based on our studies to illustrate general concepts. Since sulfur amino acids are readily transformed during food processing and participate in complex metabolic transformations *in vivo*, I first briefly summarize findings on the biological utilization and safety of the naturally occurring sulfur amino acids cysteine, methionine, and several derivatives and on the relative reactivities of the SH and the NH_2 groups, both of which are present in sulfur amino acids, to provide a chemical and biochemical basis for our studies in food safety. This summary is followed by a description of the molecular basis of reducing deleterious effects of mycotoxins, lysinoalanine, food browning, and soybean inhibitors of digestive enzymes and for improvement in food quality and health. The references cited offer the reader an entry into the comprehensive, but widely scattered, relevant literature which covers the pervasive role of sulfhydryl compounds in nature. In general, a better understanding of the molecular mechanisms underlying actions of potentially toxic and health-promoting food ingredients should facilitate the development of better and safer foods.

NOMENCLATURE

To help minimize the confusing nomenclature for sulfur amino acids used in the literature, I would like to define the following terms:

(a) *thiol*, a noun, a substance containing the sulfhydryl group, -SH, e.g., cysteine, $HSCH_2CH(NH_2)COOH$, (often used as an adjective);

(b) *sulfhydryl*, referring explicitly to the SH group;

(c) *mercapto*, adjective prefix, containing the SH group as a substituent;

(d) *thio*, adjective prefix, containing sulfur as a substituent, usually replacing oxygen;

(e) oxidized forms of SH groups

(1) *disulfide*, -S-S-, e.g., cystine,

(2) *disulfide monoxide*, -SO-S, e.g., cystine monoxide,

(3) *sulfenic acid*, -SOH, e.g., cysteine sulfenic acid,

(4) *sulfinic acid*, $-SO_2H$, e.g., cysteine sulfinic acid,

(5) *sulfonic acid*, $-SO_3H$, e.g., cysteine sulfonic or cysteic acid;

(f) *sulfide*, R-S-R, sulfur with two substituents other than hydrogen, e.g., methionine;

(g) oxidized forms of sulfides

(1) *sulfoxide*, R-SO-R, e.g., methionine sulfoxide,

(2) *sulfone*, $R-SO_2-R$, e.g., methionine sulfone;

(h) *persulfide*, R-S-SH, equivalent to peroxide, e.g., cysteine persulfide.

For conciseness, SH is used in place of sulfhydryl, mercapto, or thiol.

BIOLOGICAL UTILIZATION AND SAFETY OF SULFUR AMINO ACIDS

Only nutritious sulfur amino acids should be used to enhance the quality and safety of the diet. The following is brief summary of the biological utilization of essential and nonessential sulfur amino acids that may be present in the diet. Some of these may not be safe to consume. Figure 1 depicts the complex transsulfuration pathways that have been proposed for some of the metabolic transformations of sulfur amino acids (Friedman and Gumbmann, 1984; Reichl, 1989; Smolin and Benevenga, 1989).

Cysteine and Derivatives. Most amino acids important in nutrition have at least one asymmetric carbon atom and can exist in two mirror image (D and L) configurations, called enantiomers. Only from L-amino acids does enzyme-catalyzed polymerization build functional and structural peptides and proteins. D-Amino acids are not utilized as protein building blocks. Nevertheless, they exist in nature in both animals and plants. L-Amino acid residues in food proteins are racemized to D-isomers under the influence of acids, bases, and heat, all of which are factors common in both home and commercial food processing. Such changes may impair the nutritive quality and safety of foods by generating nonmetabolizable or nonutilizable forms of amino acids, by creating D-L, L-D, and D-D peptide bonds inaccessible to proteolytic enzymes, and by forming toxic and nutritionally antagonistic compounds.

Because sulfur and other D-amino acids may become part of our diet, a need exists to assess their biological utilization and safety and to devise methods to minimize their formation.

Using growth assays on a synthetic amino acid diet fed to mice, Friedman and Gumbmann (1984) showed that substituting D-methionine for the L-isomer resulted in a weight gain reaching approximately 76% when D-methionine was fed at a level equivalent to that optimal for the L-form (Tables 1 and 2). L-Cysteine and L-cystine stimulated growth in the presence of suboptimal levels of

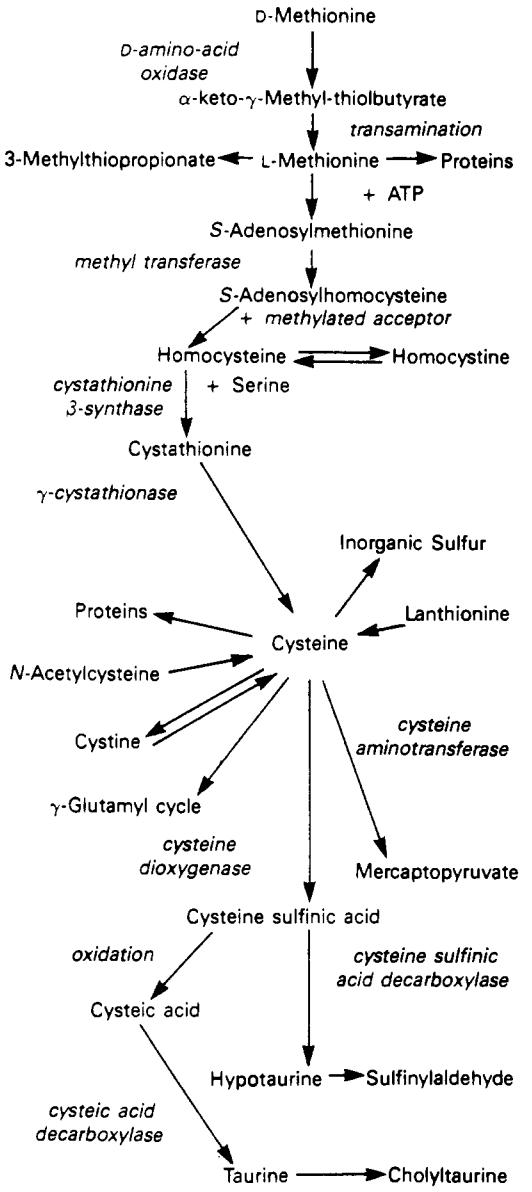


Figure 1. Transsulfuration and transamination metabolic pathways of sulfur amino acids (Friedman and Gumbmann, 1984).

Table 1. Bioavailability in Mice of L-Cysteine and Derivatives without and with Suboptimal Level of L-Methionine (Friedman and Gumbmann, 1984)

compd added	gain rel to methionine	compd added	gain rel to methionine
none	100	DL- + *meso*-lanthionine	127
L-cysteine	178	*N*-acetyl-L-cysteine	214
D-cysteine	76	L-cysteic acid	154
L-cystine	202	L-cysteine sulfinic acid	113
D-cystine	71	*S*-methyl-L-cysteine	13

L-methionine, but D-cystine was growth-depressing. L-Cystine was at least as efficient in stimulating growth in the presence of D-methionine as in the presence of the L-isomer. Compared to a suboptimal (25% of maximum) level of L-methionine alone, supplements to this level L-methionine by *N*-acetyl-L-cysteine produced a weight gain of 214%; L-cysteine, 178%; L-cysteic acid, 154%; DL- + *meso*-lanthionine, 127%; L-cysteinesulfinic acid, 113%; D-cysteine, 76%; and *S*-methyl-L-cysteine, 13%. The observed growth-depressing effect of D-cysteine, D-cysteine, and *S*-methyl-L-cysteine implies that these three sulfur-containing amino acids may be toxic. The biological utilization of D-amino acids seems to be strongly dose-dependent.

Table 2. Relative Potencies of L-Methionine Analogs and Peptides as a Nutritional Source of L-Methionine in Mice (Friedman and Gumbmann, 1988)

compd	rel potency (%) L form	rel potency (%) D form	methionine peptides (%)
methionine	100	73.1	
methionine sulfoxide	85.4	28.7	
methionine sulfone	0.0	0.0	
N-acetylmethionine	89.1	23.7	
N-acetylmethionine sulfoxide	58.7	1.7	
N-formylmethionine	86.8		
methionine, hydroxy analog Ca salt	55.4	85.7	
L-methionyl-L-methionine			99.2
L-methionyl-D-methionine			102.2
D-methionyl-L-methionine			82.1
D-methionyl-D-methionine			41.5

Our studies revealed that *N*-acetyl-L-cysteine is an excellent source of L-cysteine *in vivo*; i.e., the ability of the *N*-acetyl derivative to substitute for L-methionine for mice turned out to be even better than that of L-cysteine itself (Friedman and Gumbmann, 1984, 1988). *N*-Acetyl-L-cysteine is also well-utilized by humans (De Bernardi di Valserra et al., 1989).

A related aspect also deserves comment. The use of L-cysteine in parenteral solutions containing mixtures of amino acids is an important, but as yet unsolved, problem. Because the SH group of L-cysteine is easily oxidized and interacts with trace minerals, it is largely unstable in such solutions used to feed sick people. Efforts to overcome this problem by use of cysteine precursors and related aspects of sulfur amino acid analysis, metabolism, and nutrition are comprehensively covered by Borg and Wahlstrom (1989), Cochran and Boehm (1992), Friedman et al. (1979), Magnusson et al. (1989), Malloy et al. (1983), Neuhauser-Berthold (1989), Rassin (1989), and Sanchez and Hubbard (1989).

Methionine and Derivatives. The low content of the essential amino acid L-methionine in many food proteins of plant origin limits their nutritive value. These include soybeans and other legumes. The problem is further compounded for two reasons. First, during food processing and storage, L-methionine and other amino acids are chemically modified, further reducing nutritional quality. In the case of methionine, such modifications include oxidation to methionine sulfoxide and methionine sulfone, racemization to D-methionine, and degradation to compounds with undesirable flavors. Second, protein-bound methionine in some plant foods is poorly utilized, presumably because of poor digestibility (Begbie and Pusztai, 1989).

Another related aspect is the widespread use of L-methionine to fortify low-methionine foods to improve nutritional quality. Because of the reported antinutritional or toxic manifestations of high levels of free methionine in the diet (Figure 2), a need exists to determine whether methionine analogs and derivatives lack the apparent toxicity of L-methionine and whether they can be used as methionine substitutes in the diet. As part of a program to evaluate the nutritional and toxicological potential of novel amino acids formed during food processing, Friedman and Gumbmann (1988) compared weight gain in mice fed amino acid diets containing methionine and 16 methionine derivatives and analogs at graded dietary concentrations (Table 2). At concentrations below those yielding maximum growth, the response was close to linear. Derivatization of L-methionine generally lowered potency, calculated as the ratio of the slopes

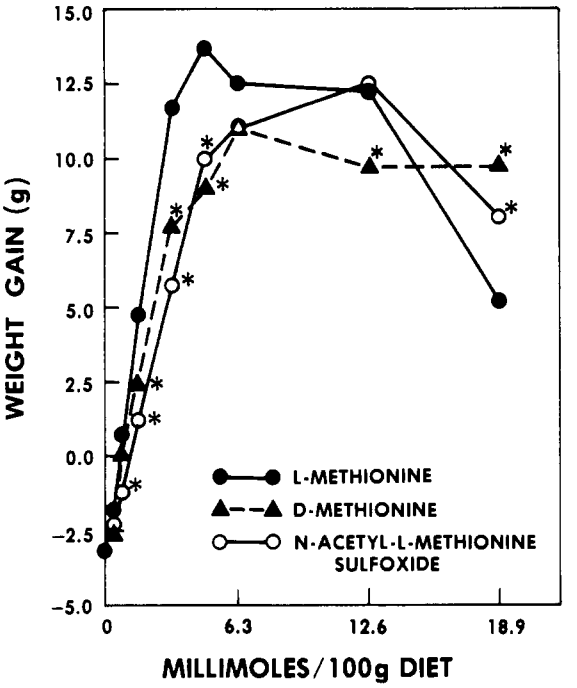


Figure 2. Weight gain in mice fed increasing dietary levels of L- and D-methionine, and *N*-acetyl-L-methionine sulfoxide for 14 days (Friedman and Gumbmann, 1988). Published 1988 by American Institute of Nutrition.

of the two dose–response curves. However, the three isomeric dipeptides L–L-, L–D-, and D–L-methionylmethionine, *N*-acetyl- and *N*-formyl-L-methionine, L-methionine sulfoxide, and D-methionine were well-utilized. *N*-acetyl-L-methionine sulfoxide reduced potency below 60%. D-Methionine sulfoxide, *N*-acetyl-D-methionine, and D-methionyl-D-methionine had potencies between 4 and 40%. The calcium salts of L- and D-α-hydroxy analogs of methionine had potencies of 55.4 and 85.7%, respectively. Several of the analogs were less growth-inhibiting or toxic at high concentrations in the diet than was L-methionine. These results imply that some methionine dipeptides or analogs may be better candidates for fortifying foods than L-methionine.

Sulfur Amino Acids and Pancreatic Function. The role of sulfur amino acids in soybean nutrition has been widely studied. A general agreement seems to have evolved on the following aspects. (a) The biological or nutritional availability of both cyst(e)ine and methionine in raw soybean meals is much less than in heated meals. This is ascribed to poor digestibility. (b) Fortification of raw or heated soybean meal with methionine significantly improves nutritional quality. The improvement is greater with raw than with heated soy products.

Sulfur amino acids also may play an important role in the feedback mechanism that has been proposed to explain the adverse effects of soy trypsin inhibitors on the pancreas. According to this hypothesis, complexation in the intestinal tract between proteolytic enzymes (trypsin, chymotrypsin) and enzyme inhibitors (Kunitz- and Bowman–Birk-type) creates a deficiency of proteolytic enzymes. This deficiency triggers an endocrine sensing mechanism which in turn induces increased protein synthesis in the pancreas. In this chain of events, undenatured trypsin inhibitors and proteolytic enzymes (both rich in sulfur amino acids) secreted by the pancreas are lost in the form of tightly bound enzyme–inhibitor complexes. This loss of sulfur amino acids is predicted to be an important modulating factor for the pancreas, which would already be under

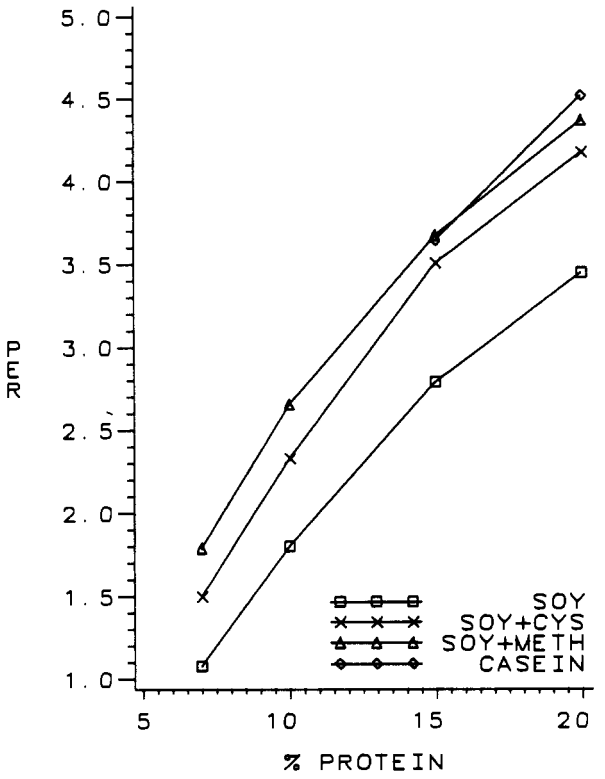


Figure 3. Effect of supplementing raw soy flour with 0.3% L-cystine, 0.3% L-methionine, or casein on the nutritional quality of the proteins as measured by the protein efficiency ratio (PER) (Gumbmann and Friedman, 1987). Published 1987 by American Institute of Nutrition.

hormonal stimulation to increase synthesis of protein especially rich in sulfur amino acids (Liener and Kakade, 1980).

In an effort to develop a better understanding of the role of sulfur amino acids in soybean nutrition, Gumbmann and Friedman (1987) found that diets containing raw soy flour fortified with L-cystine or L-methionine resulted in significant improvement in nutritional quality and in heavier rat pancreata than control diets without added sulfur amino acids (Figure 3). These results may reflect the fact that the pancreas has preferential ability over other body organs to mobilize sulfur amino acids needed for pancreatic growth.

RELATIVE REACTIVITIES

Amino (NH_2) and sulfhydryl (SH) groups in amino acids and proteins may react concurrently with a large number of structurally different electrophilic compounds. We (Cavins and Friedman, 1967, 1968, 1970; Friedman, 1968, 1972, 1977a,b; Friedman and Noma, 1970, 1986; Friedman and Tillin, 1970, 1974; Friedman and Wall, 1966; Friedman et al., 1965, 1970, 1973a, 1974, 1980; Krull and Friedman, 1967, 1969; Krull et al., 1971; Masri and Friedman, 1988) investigated factors that govern nucleophilic reactivities of functional groups in amino acids, peptides, and proteins with α,β-unsaturated compounds such as acrylonitrile, methyl acrylate, vinyl ketones, vinyl phosphonates, vinylpyridines, vinylquinolines, vinyl sulfones, and dehydroalanine. The ratio of reaction rates of SH to that of NH_2 groups varied by as much as 5000 (Tables 3 and 4). Sulfhydryl groups attached to primary carbon atoms in compounds such as cysteine and reduced glutathione reacted more rapidly than corresponding groups attached to secondary or tertiary carbon atoms. For example, the SH group of penicillamine is much less reactive with the

Table 3. Rate Ratios for Reaction of Mercaptide and Amino Groups with Acrylonitrile at pH 8.1 and 30 °C (Friedman et al., 1965)

compd	rate ratio (S^-/NH_2)	compd	rate ratio (S^-/NH_2)
DL-homocysteine	285	L-cysteine ethyl ester	1390
glutathione (reduced)	470	penicillamine	6.4
L-cysteine	304	β-mercaptoisoleucine	8.9

Table 4. Rate Ratios for Reaction of *N*-Acetyldehydroalanine Methyl Ester with the ϵ-NH_2 Group of *N*-α-Acetyl-L-lysine and with the SH Group of L-Cysteine as a Function of pH (Snow et al., 1976)

pH	rate ratio (SH/NH_2)	pH	rate ratio (SH/NH_2)
7.0	5000	10.0	133
8.0	2300	11.0	43
9.0	410	12.0	34

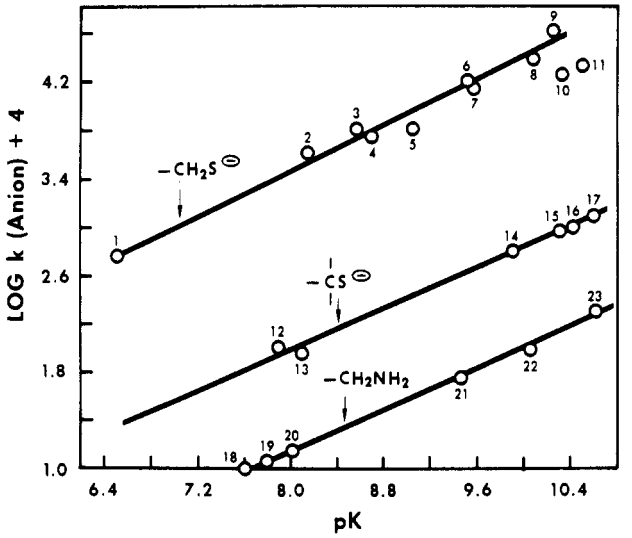
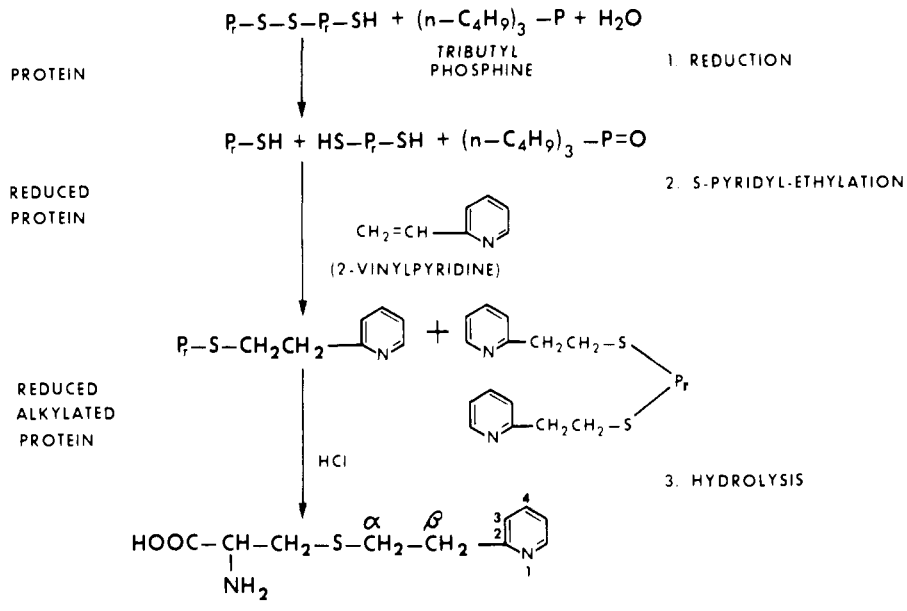


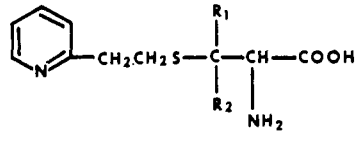
Figure 4. Plot of log second-order rate constants vs micro- and macroscopic p*K* values for the reaction of RS^- (1–17) and NH_2 groups (18–23) with acrylonitrile at 30 °C: (upper plot) mercaptide groups attached to primary carbon atoms; (middle plot) mercaptide groups attached to tertiary carbon atoms; (lower plot) amino groups attached to primary carbon atoms (Friedman, 1972).

double bond of dehydroalanine than the corresponding group in cysteine (Snow et al., 1975a,b, 1976). The observed reaction rates were correlated by linear free energy relationships in terms of structural, nucleophilic, and electronic parameters associated with the reactants (Figures 4–6). Such correlations are not only of theoretical interest as fundamental contributions to amino acid and protein chemistry but also of practical value. Our studies facilitate (a) a selective modification of protein SH groups, (b) selective modification of protein ϵ-NH_2 groups as a measure of chemically reactive and nutritionally available lysine, (c) studies of the influence of individual amino acid residues on the structure and function of proteins, and (d) the design of experiments for the chemical modification of food and other proteins to produce industrially useful materials.

Our analysis of factors governing reactivities of α,β-unsaturated compounds of structure $CH_2{=}CH{-}X$, where X is an electron-withdrawing functional group, is also relevant to recent observations that the induction of phase 2 detoxifying enzymes such as quinone reductase by anticarcinogens is due to the fact that these compounds contain the α,β-unsaturated moiety (Fernandes and Prochaska, 1993; Prochaska et al., 1992; Friedman, 1980). My hypothesis is that relative reactivities of potential

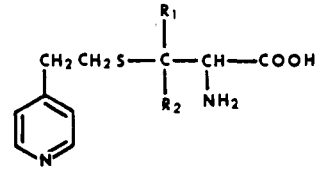


S-β-(2-PYRIDYLETHYL)-L-CYSTEINE (2-PEC)



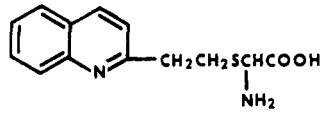
$R_1 = R_2 = H$; S-β-(2-PYRIDYLETHYL)-L-CYSTEINE (2-PEC)

$R_1 = R_2 = CH_3$; S-β-(2-PYRIDYETHYL)-DL-PENICILLAMINE

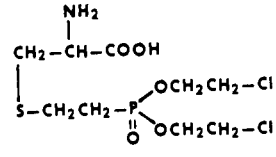


$R_1 = R_2 = H$; S-β-(4-PYRIDYLETHYL)-L-CYSTEINE (4-PEC)

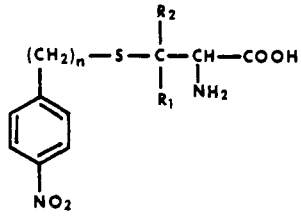
$R_1 = R_2 = CH_3$; S-β-(4-PYRIDYLETHYL)-DL-PENICILLAMINE



S-β-(2-QUINOLYLETHYL)-L-CYSTEINE (2-QEC)



S-β-bis(β′-CHLOROETHYL) PHOSPHONYLETHYL-L-CYSTEINE



n=1; $R_1 = R_2 = H$; S-β-(p-NITROBENZYL)-L-CYSTEINE

n=1; $R_1 = R_2 = CH_3$; S-β-(p-NITROBENZYL)-DL-PENICILLAMINE

n=2; $R_1 = R_2 = H$; S-β-(p-NITROPHENETHYL)-L-CYSTEINE

n=2; $R_1 = R_2 = CH_3$; S-β-(p-NITROPHENETHYL)-DL-PENICILLAMINE

Figure 5. Structures of cysteine and penicillamine derivatives. Published 1972 (lower portion) and 1980 (upper portion) by Academic Press.

carcinogens *in vitro* may predict relative potencies *in vivo*. Experimental studies are needed to verify this hypothesis.

Scheme 1 illustrates some of the possible reactions in which penicillamine, an SH-containing amino acid used to treat metal ion toxicity, can participate (Friedman, 1977b). Analogous pathways can be written for other SH-containing compounds such as *N*-acetyl-L-cysteine. Figure 5 depicts some of the synthesized cysteine and penicillamine derivatives.

MYCOTOXINS

Aflatoxins are carcinogenic polycyclic furanoid lactone (coumarone) derivatives produced by various mold species (*Aspergillus flavus, Aspergillus parasiticus*).

Aflatoxin B_1 (AFB_1) is a precarcinogen that is transformed *in vivo* to an active epoxide. Prior treatment with a site-specific reagent should modify AFB_1 in a manner that will prevent formation of the epoxide and suppress its mutagenic and carcinogenic activity. Because thiols

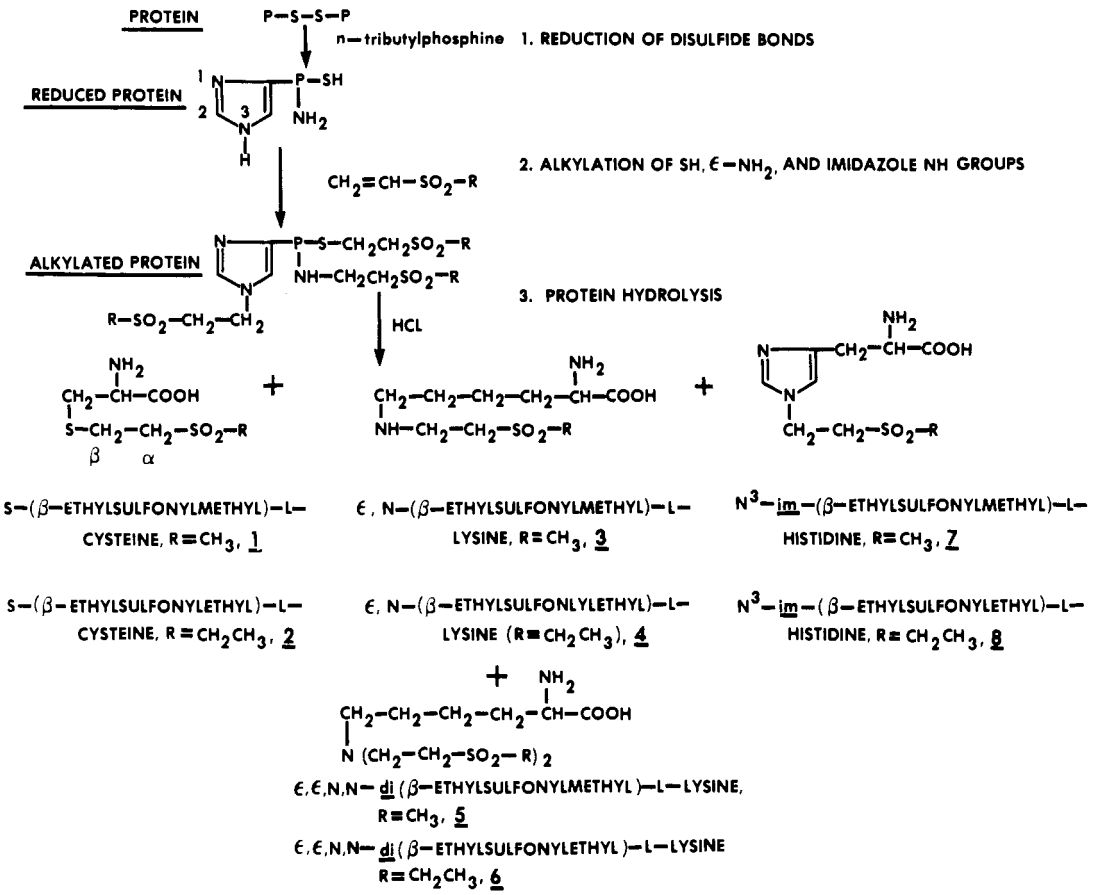


Figure 6. Alkylation of protein functional groups by methyl and ethyl vinyl sulfone (Masri and Friedman, 1988). Published 1988 by Plenum Press.

Scheme 1. Reactions of Penicillamine with Sulfur Amino Acids and Dehydroproteins

$$PSH + RSOH \rightleftarrows PSSR + H_2O \quad (1)$$
$$2PSH + RSOH \rightleftarrows PSSP + RSH + H_2O \quad (2)$$
$$4PSH + RSO_2H \rightleftarrows 2PSSP + RSH + H_2O \quad (3)$$
$$PSH + RSO_2H \rightleftarrows PSSOR + H_2O \quad (4)$$
$$PSSOR + 2PSH \rightleftarrows 2PSSR + 2PSSP + H_2O \quad (5)$$
$$2PSH + RSSOR \rightleftarrows RSSR + PSSP + H_2O \quad (6)$$
$$4PSH + RSOSOR \rightleftarrows RSSR + 2PSSP + 2H_2O \quad (7)$$
$$2PSH + MetSO \rightleftarrows Met + PSSP + H_2O \quad (8)$$
$$PSH + CH_2{=}CHPr \rightleftarrows PSCH_2CHPr \quad (9)$$

PSH = penicillamine
PSSP = oxidized penicillamine
PSSR = penicillamine–cysteine disulfide
RSOH = cysteine sulfenic acid
RSO_2H = cysteine sulfinic acid
RSSR = cystine
RSSOR = cystine monoxide
RSOSOR = cystine dioxide
Met = methionine
MetSO = methionine sulfoxide
$CH_2{=}CHPr$ = dehydroprotein
$PSCH_2CHPr$ = protein adduct

are potent nucleophiles both *in vitro* and *in vivo* (Friedman et al., 1965), they may be expected to react with electrophilic sites of AFB_1 and thus competitively inhibit the interaction of these sites with DNA or other biomolecules (Figure 7). The resulting products are, therefore, expected to be inactive in the Ames *Salmonella typhimurium* mutagenicity test and *in vivo*.

The main object of our study (Friedman et al., 1982c) was to investigate inhibition of mutagenic activity of AFB_1 in the Ames *S. typhimurium* test by thiols as a function of thiol concentration, pH, and time of treatment. We were especially interested in *N*-acetyl-L-cysteine (NAC), which has certain advantages over other thiols, such as cysteine. For instance, the SH group of NAC has a higher p*K* than the corresponding group in cysteine (9.5 vs 8.3). The ionized SH group of NAC is therefore a better nucleophile than the corresponding group in cysteine; it is also more stable to oxidation. In addition, the acetylated NH_2 in NAC, in contrast to the free NH_2 of cysteine, cannot itself react with AFB_1.

Table 5 shows results of our comparison of inactivation by 12 different thiols. Several of these effectively suppressed the mutagenic activity of AFB_1. L-Cysteine was not a very potent inactivator under our conditions. This comparison was then used as the basis for a kinetic study with the three most effective thiols: NAC, reduced glutathione (GSH), and *N*-mercaptopropionylglycine (MPG). The results in Figure 8 show the trends in aflatoxin inactivation by these three thiols. Inactivation can be accomplished over a wide range of thiol concentrations. These three thiols appear to be equally effective at the higher concentrations, but GSH appears to be more effective at the lower levels.

High-performance liquid chromatography (HPLC) studies showed that the disappearance of AFB_1 was accompanied by the appearance of a single new peak on the chromatogram. The integrated UV absorbance of this peak indicated that AFB_1 was converted nearly quantitatively to this single derivative, presumably the *N*-acetylcysteine adduct.

Related studies (Calhoun et al., 1989; Hill and Camp, 1979; Kupchan, 1974) demonstrated that the conjugated system of sesquiterpene lactones such as parthenin and hymenoxon and possibly of T-2 toxins (Friedman, 1984b) can participate with thiols in addition reactions analogous to those described for AFB_1.

Our observations may be useful in treating aflatoxin toxicity. Any agent that would compete successfully *in vivo* at DNA sites for aflatoxin may be useful in treating aflatoxin poisoning. Thus, treatment of poisoned animals and humans with *N*-acetyl-L-cysteine may minimize or

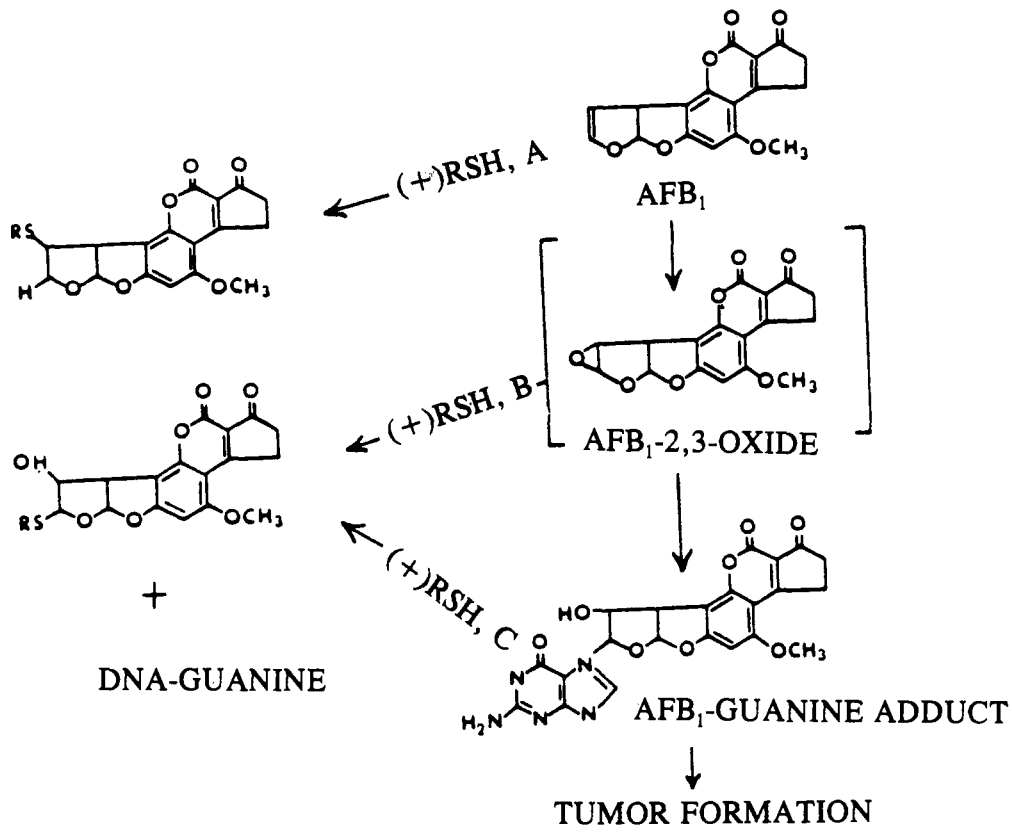


Figure 7. Some possible aflatoxin–thiol interactions: (pathway A) interaction of the 2,3-double bond of aflatoxin B_1 to form an inactive thiol adduct; (pathway B) interaction of a thiol with AFB_1-2-3-epoxide, which may prevent the epoxide from interacting with DNA; (pathway C) displacement of an aflatoxin–DNA (guanine) adduct blocking tumorigenesis.

Table 5. Inactivation of Aflatoxin B_1 Mutagenicity by Various Thiols (Friedman et al., 1982c)

compd	% original activity	p*K*
N-acetyl-L-cysteine (NAC)	0	9.5
mercaptopropionic acid	<0.2	10.4
mercaptoethanol	<0.3	9.6
reduced glutathione (GSH)	<0.6	8.8
N-2-mercaptopropionylglycine (MPG)	<2.6	10.4
mercaptoacetic acid	7	10.7
mercaptosuccinic acid	70	10.9
L-cysteine	79	8.3
acetyl-D,L-homocysteine thiolactone	85	
cysteine methyl ester	85	6.6
D-penicillamine	94	7.9
β-mercaptoethylamine (cysteamine)	100	8.3

prevent the toxic (hepatocarcinogenic) effects of aflatoxin B_1. Thiols may also be useful prophylactic agents (Mandel et al., 1993; Shetty et al., 1989).

LYSINOALANINE

Alkali treatment of food proteins has been used for many purposes. These include preparing meat analogs from vegetable (soy) protein, destroying aflatoxin, peeling fruits and vegetables, and preparing protein concentrates (Friedman et al., 1984b,c).

Cross-linked amino acids have been detected in alkali- and heat-treated proteins (Bellomonte et al., 1987). One of these cross-linked derivatives of lysine, lysinoalanine, has been found to cause histological changes in the descending portion (*pars recta*) of the proximal tubules of rat kidneys. The lesions are characterized by enlargement of the cell nucleus (karyomegaly), increased nucleoprotein content, and disturbances in DNA synthesis and mitosis (Anonymous, 1989; Friedman et al., 1982b; Gould and MacGregor, 1977; Karayiannis et al., 1979a,b; Langhendries et al., 1992; Woodard et al., 1975).

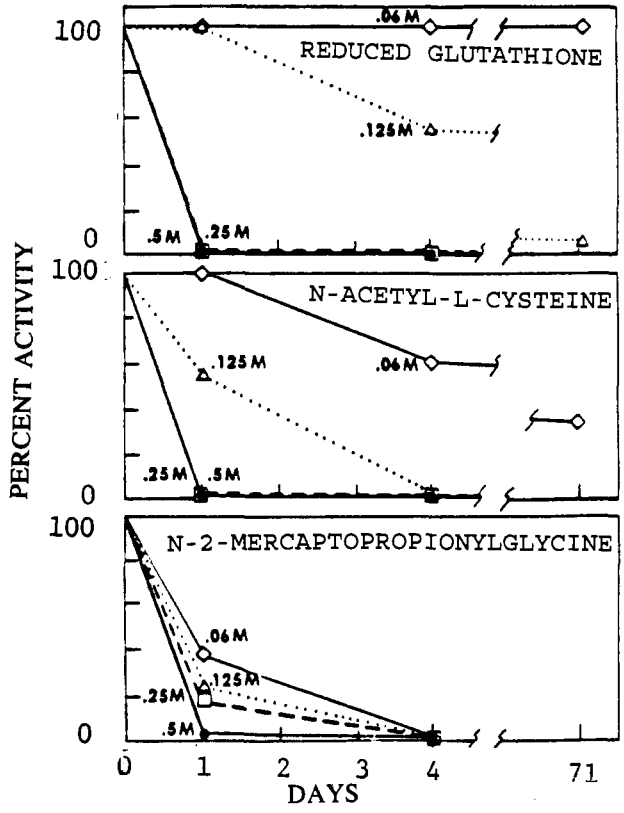


Figure 8. Effect of thiol concentration on time of inactivation of mutagenic activity of aflatoxin B_1 by reduced glutathione, *N*-acetylcysteine, and *N*-2-mercaptopropionylglycine (Friedman et al., 1982c).

The apparent direct relationship between the observed affinities of the lysinoalanine (LAL) isomers for copper(II) ions *in vitro* and their relative toxic manifestation in

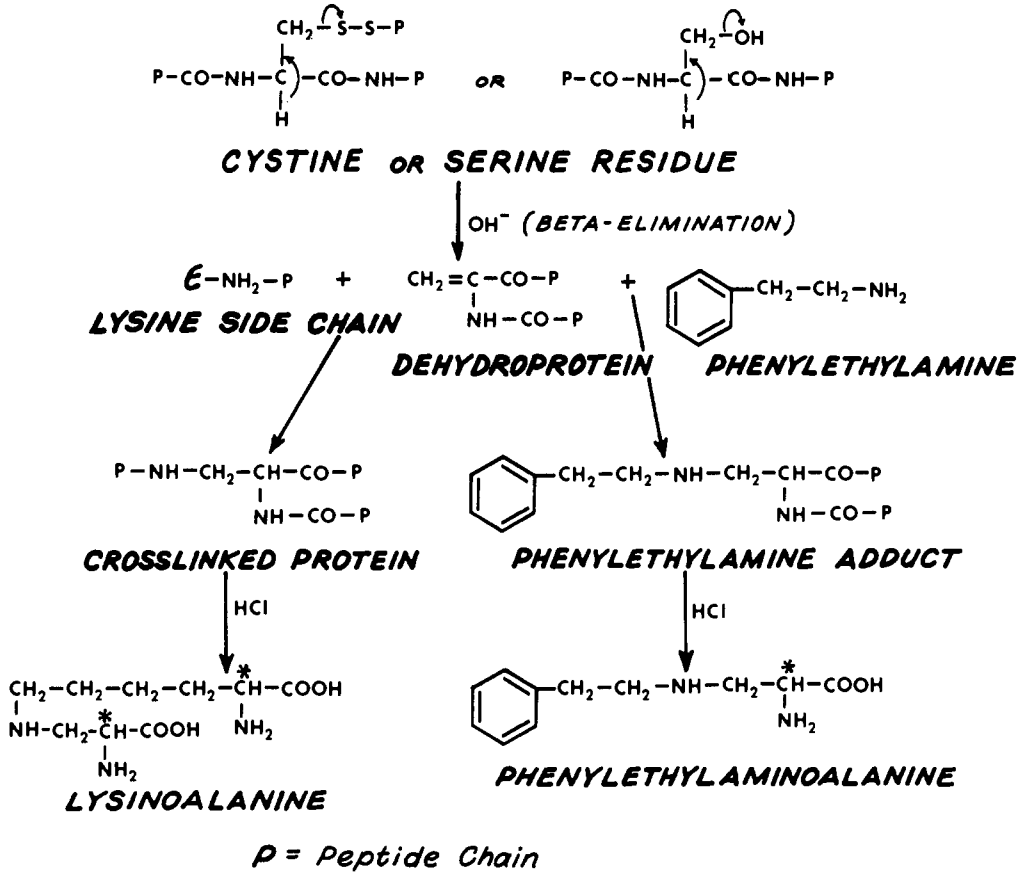


Figure 9. Transformation of cystine and serine residues in a protein to lysinoalanine and [(phenylethyl)amino]alanine. Newly created asymmetric centers are indicated by asterisks. Published 1986 by American Chemical Society.

the rat kidney is consistent with our hypothesis that LAL exerts its biological effect through chelation of copper in body fluids and tissues (Friedman, 1974, 1977a; Hayashi, 1982; Friedman et al., 1986; Pearce and Friedman, 1988; Friedman and Pearce, 1989). Limited studies on the binding of LL-and LD-lysinoalanines to cobalt(II), zinc-(II), and other metal ions imply that lysinoalanine could also influence cobalt utilization *in vivo*. Animal studies are needed to confirm the predicted role of lysinoalanine in metal ion transport, utilization, and histopathology as well as chemical studies to minimize the formation of the most toxic isomers.

These observations raise concern about the nutritional quality and safety of alkali-treated proteins. The chemical changes that produce unnatural amino acids such as lysinoalanine need to be explained, and strategies to minimize or prevent these reactions need to be developed. However, since the mechanism by which these compounds damage the rat kidney is unknown, it is difficult to assess the risk to human health caused by their presence in the diet.

The following section describes our efforts to decrease lysinoalanine formation in food proteins.

Lysinoalanine Formation. A postulated mechanism of lysinoalanine formation (Figures 9 and 10) is at least a two-step process (Friedman, 1977a, 1982a). First, hydroxide ion-catalyzed elimination reactions of serine, threonine, and cystine give rise to a dehydroalanine intermediate. Second, a dehydroalanine residue, which contains a carbon–carbon double bond, reacts with the ϵ-amino group of lysine to form a lysinoalanine cross-link. This combination is governed not only by the number of available amino groups but also by the location of the amino group and dehydroalanine potential partners in the protein chain. When convenient sites have reacted, additional lysinoalanine (or other) cross-links form less readily. Each protein, therefore, may have a limited fraction of sites for forming cross-linked residues. The number of such sites is presumably dictated by the protein's size, composition, conformation, chain mobility, steric factors, and extent of ionization of reactive amino (or other) nucleophilic centers.

Lysinoalanine Inhibition. The double bond of dehydroalanine reacted more rapidly with SH groups of cysteine than with the ϵ-NH_2 group of lysine (Table 4). Thus, depending on the pH of the reaction, sulfhydryl groups of cysteine react about 34 to 5000 times faster than the ϵ-amino groups of lysine with vinyl compounds such as *N*-acetyldehydroalanine methyl ester (Friedman et al., 1977; Snow et al., 1976). Therefore, by adding thiols such as cysteine, it may be possible to prevent the formation of dehydroalanine residues during alkali treatment of proteins (Table 6). Sodium sulfite also inhibits lysinoalanine formation (Table 7).

Mechanistic considerations suggest that added thiol or sulfite ions can inhibit lysinoalanine formation by at least three distinct mechanisms. The first is by direct competition. The added nucleophile (mercaptide, sulfite, bisulfite, cyanide, thiocyanate, thiourea, etc.) can trap dehydroalanine residues derived from protein amino acid side chains, forming their respective adducts. The second possible mechanism is best described as indirect competition. The added nucleophile can cleave protein disulfide bonds and thus generate free protein SH groups. These may then combine with dehydroalanine residues. The third possible mechanism can be described as suppression of dehydroalanine formation. The added nucleophile, by cleaving disulfide bonds, can diminish a potential source

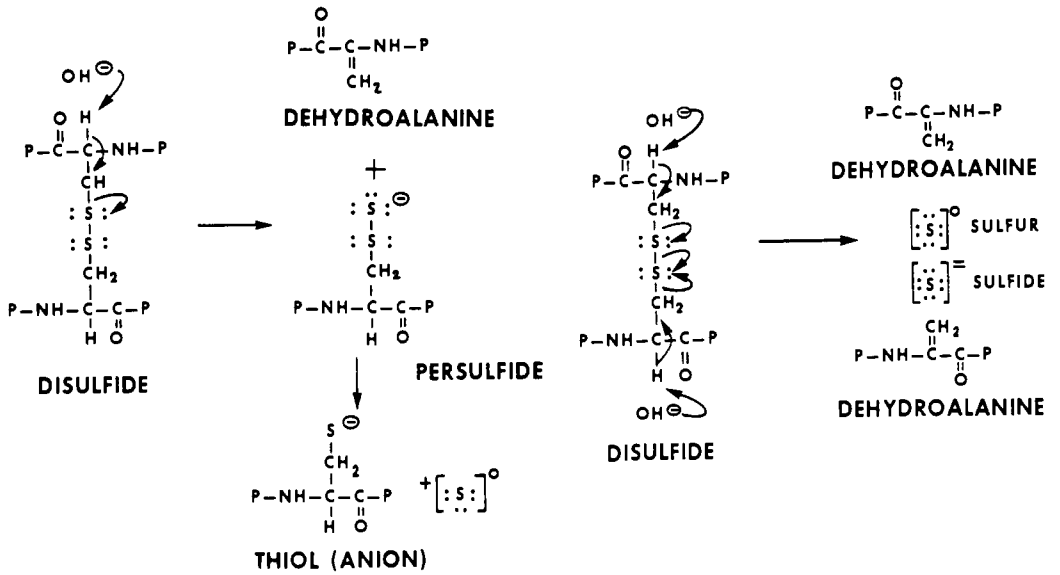


Figure 10. Mechanisms for base-catalyzed transformation of a protein–disulfide bond.

Table 6. Effect of Thiols on Lysinoalanine Content of Alkali-Treated Soy Protein (Friedman et al., 1984b)

additive	lysinoalanine wt %	lysinoalanine mol %
none	4.04	1.09
L-cysteine	1.69	0.36
N-acetyl-L-cysteine	1.72	0.38
reduced glutathione	1.05	0.38

Table 7. Lysine (LYS), Lysinoalanine (LAL), and Lanthionine (LAN) Content of Alkali-Treated Proteins (Friedman, 1977a)

protein and treatment[a]	μmol/g LYS	μmol/g LAL	μmol/g LAN
wool: untreated	222.5	0.0	0.0
pH 11.6	139.0	58.7	119.5
pH 11.6 + Na_2SO_3	176.9	19.1	0.0
casein: untreated	482.7	0.0	0.0
pH 11.6	294.0	145.2	0.0
pH 11.6 + Na_2SO_3	373.0	65.7	0.0
lysozyme: untreated	397.3	0.0	0.0
pH 11.6	222.2	135.2	0.0
pH 11.6 + Na_2SO_3	317.2	35.6	0.0
soybean trypsin inhibitor: untreated	319.3	0.0	0.0
pH 11.6	129.2	235.5	0.0
pH 11.6 + Na_2SO_3	239.0	36.8	0.0

[a] The amount of LAL formed under similar conditions varied greatly among the four proteins tested; lanthionine was formed in alkali-treated wool; the presence of Na_2SO_3 resulted in decreases in LAL formation and complete suppression of LAN formation.

of dehydroalanine, inasmuch as the resulting ionized and thus negatively charged *cysteine* undergoes elimination reactions to form dehydroalanine much less readily than the original *cystine* (disulfide) precursor residues.

The stereochemical course of addition reactions to dehydroalanines deserves comment since the reactions generate diastereoisomers with different biological properties (Friedman, 1977a; Friedman and Pearce, 1989; Liardon et al., 1991; Weisleder and Friedman, 1968). Specifically, reaction of the SH group of cysteine with the double bond of dehydroalanine gives rise to one pair of optically active lanthionine isomers (enantiomers) and one *meso* form. Addition of the SH group to methyldehydroalanine generates four isomers. Similarly, addition of the ε-NH_2 group of L-lysine to dehydroalanine produces LL- and LD-lysinoalanines. The corresponding products from D-lysine are DD- and DL-lysinoalanines. Figure 11 illustrates possible mechanisms governing these additions.

Table 6 compares the effects of several compounds on the lysinoalanine and lysine contents of alkali-treated soybean protein. These results show that all of these compounds partly inhibit lysinoalanine formation. The extent of inhibition may vary from protein to protein and should be related to both the content and reducibility of the disulfide bonds.

Lysinoalanine formation can also be inhibited by acylation of ε-NH_2 group of lysine residues in proteins, by the presence of carbohydrates, which block lysine ε-NH_2 groups, and by the presence of dimethyl sulfoxide (Friedman et al., 1984b,c).

FOOD BROWNING

Reactions of amino acids, peptides, and proteins with sugars (nonenzymatic browning) and quinones (enzymatic browning) cause deterioration of food during storage and commercial or domestic processing. The loss in digestibility and nutritional quality due to destruction of essential amino acids is accompanied by destruction of potentially anticarcinogenic polyphenolic compounds. The production of antinutritional and toxic compounds may further reduce the nutritional value and possibly the safety of foods (Finley and Friedman, 1973; Friedman, 1982b, 1991a; Friedman and Cuq, 1988; Friedman and Finot, 1990; Friedman et al., 1987; Oste and Friedman, 1990; Oste et al., 1990; Pearce et al., 1988; Smith and Friedman, 1984; Ziderman et al., 1989). Studies in this area include (a) influence of damage to essential amino acids, especially lysine, methionine, and tryptophan, on nutritional quality; (b) attempts to restore nutritional quality by fortifying browning products with essential amino acids; (c) nutritional damage as a function of processing conditions; (d) biological utilization of characterized browning products; and (e) formation of food toxicants including kidney-damaging compounds, growth inhibitors, mutagenic (DNA-damaging), clastogenic (chromosome-damaging), and carcinogenic compounds (Friedman et al., 1990; Gumbmann et al., 1983; Lee et al., 1982; McGregor et al., 1989).

Sulfites have been used to inhibit enzymatic and nonenzymatic browning reactions (Wedzicha et al., 1991). Sulfite substitutes are needed since sulfites are reported to induce asthmatic crises in 4–8% of exposed asthmatics, and their use is being discontinued. Our studies show

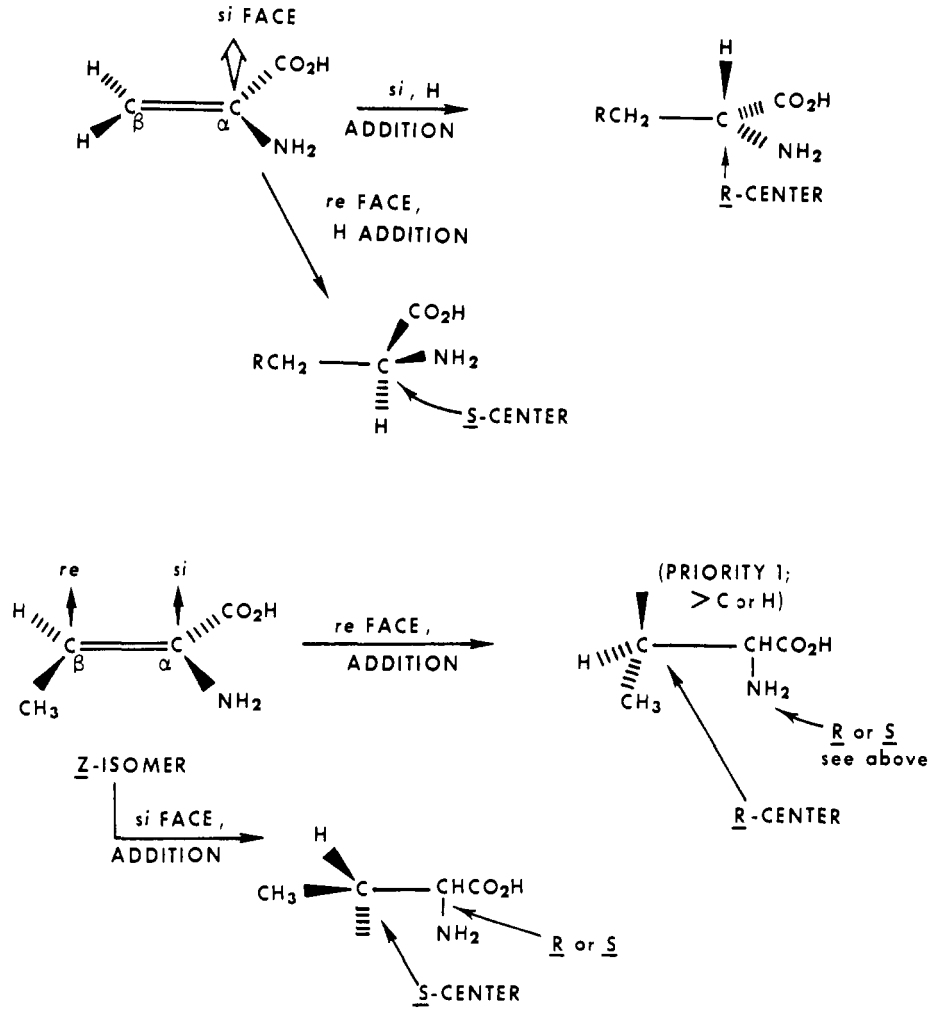


Figure 11. (Upper plot) Stereochemical course of proton addition to the α-carbon of dehydroalanine. When R is a sulfur atom, the change in priorities (S > O) reverses the configuration. (Lower plot) Stereochemical course of addition to β-carbon of 3-methyldehydroalanine, where X = ligand (S, O, N). The stereochemistry at the β-center will be the same for the *E*-isomer; i.e., *re* addition of X at β-carbon gives *R* center at β-carbon, and *si* addition of X gives *S* center at β-carbon. However, *re*(β-C)–*si*(α-C) addition of X and H is a *syn* addition with *Z*-isomer; whereas *re* (β-C)-*si*(α-C) addition of X and H is an *anti* process with *E*-isomer.

Table 8. Prevention of Browning in Commercial Fruit Juices by Sodium Bisulfite and *N*-Acetyl-L-cysteine (Molnar-Perl and Friedman, 1990a)

	inhibition (%)									
	sodium bisulfite (mM)					*N*-acetyl-L-cysteine (mM)				
juice	1.0	2.0	4.0	8.0	16.0	2.5	1.0	25	50	100
grape	10	25	69	72	100	6	18	35	79	100
apple	42	50	59	93	100	1	36	52	87	100
apple	10	40	49	100	100	5	40	55	92	93
pineapple	11	39	68	102	105	10	35	54	76	100
grapefruit	18	25	39	63	100	32	61	75	87	93
orange	13	49	69	92	108	3	69	66	72	107

Table 9. Prevention of Browning in Protein-Containing Foods by Sodium Bisulfite and *N*-Acetyl-L-cysteine (Molnar-Perl and Friedman, 1990a)

	inhibition (%)									
	sodium bisulfite (mM)					*N*-acetyl-L-cysteine (mM)				
protein source	2.5	25	50	100	200	25	6.2	125	250	500
casein	4	12	44	82	100	0	25	42	101	101
barley flour	3	43	61	98	95	36	42	79	96	104
soy flour	10	27	80	98	102	19	38	84	99	101
nonfat dry milk	3	23	44	94	104	19	43	78	98	101
Isomil	7	29	72	88	100	7	43	65	93	109

that certain sulfur amino acids, such as *N*-acetylcysteine and the natural peptide glutathione, are nearly as effective as sulfite in preventing browning of a wide variety of foods, including heated protein–sugar mixtures, apples, potatoes, and fruit juices. These sulfur amino acids merit further study to assess their potential for preventing long-term food browning under practical storage and processing conditions. Studies are also needed to determine whether these sulfur amino acids can prevent the formation of heterocyclic amines (**IQ** compounds), potent carcinogens formed during food processing (de Flora et al., 1989; Weisburger, 1991).

To demonstrate whether SH-containing sulfur amino acids minimize nonenzymatic browning, a mixture of amino acids was heated with glucose in the absence and presence of the following potential inhibitors: *N*-acetyl-L-cysteine, L-cysteine, reduced glutathione, sodium bisulfite, and urea (Friedman and Molnar-Perl, 1990; Friedman et al., 1992; Molnar and Friedman, 1990a,b). Inhibition was measured as a function of temperature, time of heating, and concentration of reactants. The extent of browning was estimated by absorbance measurements at 420 nm. The minimum concentrations for optimum inhibition, in moles of inhibitor per mole of D-glucose, were as follows: sodium bisulfite, 0.02; L-cysteine, 0.05; *N*-acetyl-L-cysteine, 0.2; reduced glutathione, 0.2; urea, 8. An "index of prevention" (IP) was used to calculate the inhibition at the optimum mole ratio range (Tables 8 and 9; Figure 12).

Although the nature of the inhibition processes is not well understood, possibilities include (a) suppression of free-radical formation, whereby the formed radicals during

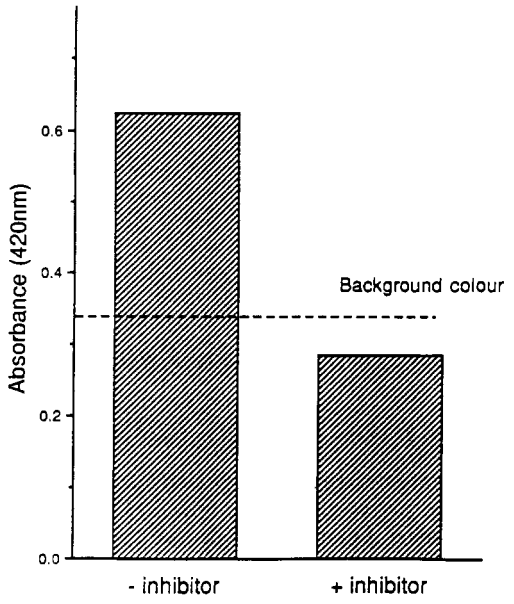


Figure 12. Inhibition of potato browning by *N*-acetyl-L-cysteine (Friedman et al., 1992b). Published 1992 by Taylor and Francis.

heating are abstracted by and localized on the sulfur moiety of the thiol; and (b) interaction of the sulfhydryl compounds with intermediates formed during browning product(s). Because of their strong nucleophilic reactivity and ability to dissipate free radicals, sulfur amino acids are especially capable of participating in the cited transformations on the basis of their extensively studied chemical properties (Cilliers and Singleton, 1990; Dudley and Hotchkiss, 1989; Friedman et al., 1987; Golan-Goldhirsh and Whitaker, 1984).

Future studies should emphasize the prevention of browning and the consequent antinutritional and toxicological manifestations of browning products in whole foods as consumed. Many of the safety concerns cited, especially those of genotoxic potential, are based on *in vitro* data which may not always be relevant to *in vivo* effects following the consumption of whole food products containing the browning-derived constituents. The presence of other dietary constituents in the food and the process of digestion and metabolism can be expected in some cases to decrease or increase the adverse manifestations of browning products.

SOYBEAN INHIBITORS OF DIGESTIVE ENZYMES

Inhibitors of enzymes such as trypsin, chymotrypsin, carboxypeptidase, elastase, and α-amylase appear in many agricultural products including legumes, cereals, and potatoes (Domagalski et al., 1992; Friedman and Gumbmann, 1986a,b; Friedman et al., 1982a, 1989, 1991; Liener and Kakade, 1980; Rackis et al., 1986; Weder, 1986). Soybean and other legumes containing active protease inhibitors depress growth in rats compared to analogous feeding of inhibitor-free soybeans. Growth inhibition and the accompanying pancreatic hypersecretion of trypsin and chymotrypsin, pancreatic hypertrophy, and pancreatic adenoma are presumably partly due to the antitryptic and antichymotryptic activities of the inhibitors, since toasting soy flour, which inactivates most of the inhibitors, largely prevents pancreatic enlargement and related changes (Gumbmann et al., 1986; Liener and Hasdai, 1986; Morgan et al., 1986). Although the possible significance of trypsin inhibitors for human health is yet to be resolved (Hathcock, 1991; Liener et al., 1988; Toskes, 1986; Wormsley and Roebuck, 1988), the concern, if any, could be avoided by eliminating the inhibitors from the diet.

Table 10. Half-Cystine Content of Proteins and Flours Determined as *S*-β-(4-Pyridylethyl)-L-cysteine (Friedman et al., 1970; Krull and Friedman, 1969; Wu et al., 1971)

	half-cystine (mol/10^5 g)	
protein source	ion-exchange chromatography	spectrophotometry
bovine serum albumin	49.1	50.2
wheat gluten	17.5	17.5
lysozyme	56.2	53.8
β-lactoglobulin	29.2	
ribonuclease	59.9	
ovalbumin	13.4	
defatted soy flour	13.2	
wheat flour	12.2	

Heat alone does not completely inactivate all inhibitory activity. Possible adverse effects of residual inhibitors in food are largely unknown. In addition, heat used to inactivate inhibitors may also destroy certain essential amino acids, such as cystine, methionine, and lysine. These considerations prompted us to examine a possible synergistic effect of heat and disulfide bond modification on the activity of soybean inhibitors both in pure form and in soy flour.

The following section summarizes some of our findings with three types of disulfide bond modification: reductive S-alkylation, sulfhydryl–disulfide interchange, and sulfitolysis of disulfide bonds.

S-Pyridylethylation. An ideal reagent for the selective modification of SH groups should meet the following requirements: (a) it should selectively modify the SH groups under mild conditions; (b) modified cysteine residues should survive acid hydrolysis; (c) cysteine derivatives should be eluted in a convenient position as a well-resolved peak in standard amino acid analysis; and (d) the derivative should contain a chromophore that can be determined independently, namely by ultraviolet or fluorescence spectroscopy.

Our discovery that vinylpyridines (Table 10), vinylquinoline, and *p*-nitrostyrene selectively alkylated SH groups derived from the cleavage of disulfide bonds was used to prepare a series of derivatives with partial to complete S-pyridylethylation (Cavins and Friedman, 1970; Friedman and Noma, 1970; Friedman et al., 1980; Masri and Friedman, 1982; Masri et al., 1972; Zahnley and Friedman, 1982). Table 11 shows the effect of the modification on the inhibitory activities against trypsin and chymotrypsin. Loss of activity was proportionate to the extent of modification and presumably arises from the change in the native conformation of the protein inhibitors (Figure 13).

Our studies also show the following advantages of the cited procedure for reductive alkylation of disulfide bonds in proteins: (a) the cystine content can be estimated by two independent techniques, i.e., ion-exchange chromatography and ultraviolet spectroscopy; (b) the use of ion-exchange chromatography permits estimation of the other amino acids as well as cystine in a single determination; and (c) the method is especially useful for determining the extent of partial reduction of disulfide bonds (Friedman and Tillin, 1974; Griffith, 1980).

Sulfhydryl–Disulfide Interchange. The action of thiols such as cysteine in inhibiting the activity of disulfide-containing enzyme inhibitors (Tables 12–14) is postulated to involve formation of mixed disulfides among added thiols, enzyme inhibitors, and storage proteins (Scheme 2). Since oxygen was not excluded, formation of new disulfide bonds probably proceeded by sulfhydryl–disulfide interchange (eqs 10–14) and oxygen-catalyzed oxi-

Table 11. Half-Cystine Content of Inhibitors of Digestive Enzymes Determined as *S*-β-(2-Pyridylethyl)-L-cysteine (Friedman et al., 1980)

protease inhibitor	time (min)	mol of half-cystine/ mol of protein	S–S bonds cleaved (%)	% inhibitory activity against	
				trypsin	chymotrypsin
soybean	30	4.0	100	0	
	60	4.6	100	0	
	120	4.4	100	0	
	240		100	0	
lima bean	30, no urea		14	32	19
	30, 8 M urea		40	10	15
	240, 8 M urea	7.4	51	0	0
ovomucoid	240, 8 M urea	15.3	81	0	

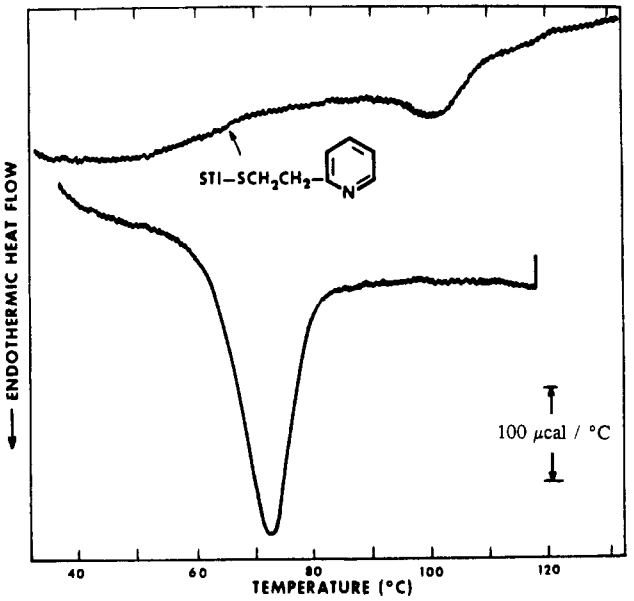


Figure 13. Differential scanning calorimetry (DSC) of soybean trypsin inhibitor (bottom scan) and its *S*-pyridylethyl derivative (top scan). The DSC thermogram of native STI shows a large endothermic peak at 73 °C. The modified protein lacks the organized structure normally disrupted at 73 °C (Friedman et al., 1982d).

dation of two SH groups to an S–S bond. One of the sulfur atoms of the mixed disulfide originated from the protein and the other from added sulfhydryl compound. The added compound, therefore, disappears and becomes part of the protein structure. Because of structural alterations due to formation of mixed disulfide, the modified inhibitors lose their ability to complex with the active sites of trypsin and other proteolytic enzymes (Jiao et al., 1992).

Evidence for the postulated reaction scheme was obtained by measuring the cysteine (SH) content of soybean flours. Table 12 shows that cysteine treatment did increase the half-cystine content, as would be expected if the added thiols had participated in the indicated sulfhydryl–disulfide interchanges. This result implies that the added cysteine or acetylcysteine (a) participated in the indicated sulfhydryl–disulfide interchange and oxidation reactions and (b) become covalently attached to soybean protein chains in the form of mixed disulfides. The thiol treatments also resulted in improvement in digestibility and nutritional value as measured by the protein efficiency ratio (PER) (Table 13; Figure 14).

Sulfitolysis of Disulfide Bonds. Heat plus sulfite may act synergistically in improving the nutritional quality and safety of soy flour as reflected in the PER values and pancreatic weights for two reasons. Sulfite ions can, in principle, also cleave protein disulfide bonds to form a thiol anion ($P\text{–}S^-$) and a *S*-sulfocysteine derivative ($P\text{–}S\text{–}SO_3^-$) by the following equations:

$$P\text{–}S\text{–}S\text{–}P + SO_3^{2-} \rightleftarrows P\text{–}S^- + P\text{–}S\text{–}SO_3^-$$

$$P\text{–}S\text{–}SO_3^- + P\text{–}S^- \rightleftarrows P\text{–}S\text{–}S\text{–}P + SO_3^{2-} \text{ (regenerated)}$$

The *S*-sulfocysteine can interact further with the generated $P\text{–}S^-$ to form a new disulfide bond and SO_3^-. The net effect is a rearrangement of protein disulfide bonds catalyzed by SO_3^{2-}.

Thus, exposure of disulfide-containing trypsin inhibitors to sulfite ions should alter their structures and their inhibitory properties in analogy with the cysteine treatments described earlier. This hypothesis was tested by exposing soy flour to 0.03 M sodium sulfite and feeding the dialyzed and lyophilized materials to rats (Friedman and Gumbmann, 1986b). The sulfite treatment at 75 °C lowered the trypsin inhibitor level in soy flour to zero. This value is usually difficult to achieve even at high temperatures without marked deleterious effects on protein quality. This finding suggests that the sulfite treatment is highly effective in inactivating trypsin inhibitors and improving the nutritional quality of soy flour.

After dialysis, the sodium-sulfite-treated soy flour contained no measurable amounts of residual sodium sulfite. This finding may alleviate concern about the use of sodium sulfite to improve the nutritional quality and safety of foods containing inhibitors. Precipitating soy proteins at their isoelectric point near pH 4.5 may be a more practical way of separating the proteins from sodium sulfite. However, this may not work for a soy concentrate or flour.

Heat significantly improved protein quality of raw soy flour. Treatment with sodium sulfite further increased the protein efficiency ratio (PER). This improvement in protein quality is also reflected in an increase in *in vivo* nitrogen digestibilities. Table 14 shows that pancreas weights were elevated in rats fed raw soy flour but not in those fed heated soy flour, with or without sodium sulfite (Gonzalez and Domadaran, 1990; Sessa and Gantous, 1987).

Beneficial Effect of Inhibitors. The reported anticarcinogenic action of the Bowman–Birk protease inhibitor of soybeans (Brandon et al., 1993; Kennedy et al., 1993) deserves special mention. Although the biochemical and molecular basis for the beneficial effect needs to be elucidated since it raises the question of how a protein can prevent cancer, one possibility is that the inhibitor–protease complexes formed *in vivo* after consumption of the inhibitor can act as free-radical traps whereby the free electrons of hydroxyl radicals inducing cancer are dissipated to sulfur atoms of the cystine-rich inhibitors or complexes. The sulfur amino acid content of the Bowman–Birk inhibitor is very high and is similar to that of keratins such as wool and human hair (Friedman and Noma, 1970; Menefee and Friedman, 1985), with 1 of every 5–10 amino acids being cystine.

Table 12. Trypsin Inhibitor, SH, and Half-Cystine Content of Treated Soy Flours (Friedman et al., 1984a)

protein source	mg of trypsin inhibited/g of soy flour	SH content (nmol/mg)	half-cystine (nmol/mg)
native soy flour	37.5	3.85	71.6
soy flour, heated at 45 °C	15.83	1.29	73.1
soy flour plus cysteine, heated at 45 °C	6.24	1.67	108.9
soy flour plus acetyl-L-cysteine, heated at 45 °C	4.76	1.32	147.5
soy flour, heated at 65 °C	8.18	1.12	59.1
soy flour plus cysteine, heated at 65 °C	2.87	0.89	86.2
soy flour plus acetylcysteine, heated at 65 °C	1.48	0.85	86.2
soy flour, heated at 75 °C	5.84	0.48	63.5
soy flour plus cysteine, heated at 75 °C	2.23	0.52	133.9

Table 13. Effect of Soy Flour Treated with L-Cysteine and *N*-Acetyl-L-cysteine on PER and Nitrogen Digestibility (Friedman et al., 1984a)

soy flour treatment			
reaction temp (°C)	amino acid added	PER	nitrogen digestibility (%)
	Experiment 1		
45	none	0.95	73.7
	cysteine	2.01	81.7
	acetylcysteine	2.20	82.7
65	none	1.61	79.9
	cysteine	2.43	82.9
	acetylcysteine	2.02	77.0
	casein control	3.05	93.3
	Experiment 2		
75	none	2.14	79.0
	cysteine	2.53	82.7
casein control		3.19	94.6
pooled standard error		±0.08	±0.7

Table 14. PER and Digestibility of Raw, Heated, and Sulfite-Treated Soy Flour (Friedman and Gumbmann, 1986b)

diet	PER	digestibility (%)		pancreas wt (% of body wt)
		diet	nitrogen	
raw soy flour	1.55	90.9	78.3	0.51
raw soy flour (dialyzed)	1.65	90.9	79.2	0.56
heated soy flour	2.11	91.3	81.9	0.43
heated soy flour (+0.03 M sulfite)	2.49	91.7	84.0	0.40
ANRC casein	3.44	94.4	93.0	0.40
SE	±0.06	±0.4	±0.7	±0.02

Scheme 2. Sulfhydryl–Disulfide Interchange and Oxidation Pathways

$$R{-}SH + In{-}S{-}S{-}In \rightleftarrows R{-}S{-}S{-}In + HS{-}In \quad (10)$$

$$R{-}SH + Pr{-}S{-}S{-}Pr \rightleftarrows R{-}S{-}S{-}Pr + HS{-}Pr \quad (11)$$

$$In{-}SH + Pr{-}S{-}S{-}Pr \rightleftarrows In{-}S{-}S{-}Pr + HS{-}Pr \quad (12)$$

$$In{-}SH + HS{-}Pr + {}^1/_2O_2 \rightleftarrows In{-}S{-}S{-}Pr + H_2O \quad (13)$$

$$In{-}SH + HS{-}In + {}^1/_2O_2 \rightleftarrows In{-}S{-}S{-}In + H_2O \quad (14)$$

R—SH = added thiol
In—S—S—In = inhibitor protein (In) disulfide bonds
Pr—S—S—Pr = storage protein (Pr) disulfide bonds

Another possibility is that the insoluble inhibitor–protease complex physically adsorbs carcinogens during passage through the digestive tract, thus preventing induction of colon cancer; i.e., the complex acts as an insoluble dietary fiber. The key question is whether the amount of inhibitor needed, for example, to prevent colon cancer will or will not at the same time have an adverse effect on the pancreas.

In summary, treatment of soy flour with SH-containing amino acids alters the structural integrity of inhibitors and prevents them from inactivating proteolytic enzymes by complex formation. The net result is an increase in the content of sulfur amino acids in sulfur-poor legumes, loss of inhibitory activity, and increased protein digestibility and nutritive value.

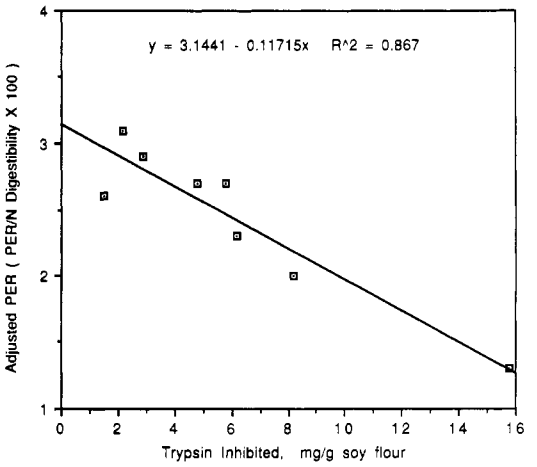


Figure 14. Linear relationship between PER adjusted for nitrogen digestibility and trypsin inhibitor content of soy flour treated with L-cysteine and *N*-acetyl-L-cysteine. Improvement in PER appears to be due mostly to decrease in trypsin inhibitor content (data from Tables 12 and 13).

FOOD QUALITY AND THERAPY

Although the impact of sulfur amino acids on food quality and their use as drugs or prodrugs are beyond the scope of this review, I will briefly mention some aspectsof these very active areas of research since they have a common molecular basis and may benefit food safety.

Food Quality. Sulfur amino acids act as precursors for the enzymatic and nonenzymatic genesis of numerous food flavors. Many of these compounds are also biologically active in animals and humans (Wargowich, 1992; Zhang and Ho, 1989). According to Schwimmer and Friedman (1972), there are at least four modes of flavor genesis in which sulfur is involved: (a) biosynthesis in foods catalyzed by enzymes before harvest, slaughter, or processing; (b) formation by action of enzymes when the integrity of the cell is destroyed; (c) genesis by action of microorganisms; and (d) formation by exposure of foods to heat, light, and ionizing radiation.

Food quality also benefits from heat-induced sulfhydryl–disulfide interchange of cysteine and cystine residues in proteins. Beneficial consequences of these and related transformations include improvement in (a) rheological properties of dough during baking (Harinder and Bains, 1988; Jackson and Hoseney, 1989; Kiefer et al., 1990), (b) cooking quality of pasta (Alary and Kobrehel, 1987), (c) digestibility of fish proteins (Opstvedt et al., 1984), and (d) gelation and other physicochemical properties of proteins from eggs (Mine et al., 1990; Nguyen and Burley, 1984), lima bean hemagglutinins (Wallace and Friedman, 1983), soybeans (Wang and Damodaran, 1990), and whey (Kella et al., 1989; Shimada and Cheftel, 1989).

The beneficial changes in food proteins are probably due to the fact that disulfide bonds are paramount in governing the three-dimensional structure of proteins.

Heat-induced rearrangement of disulfide bonds generally does not affect the amino acid composition. However, it does affect noncovalent interactions between amino acid residues. The consequences are major changes in the structure and conformation of protein chains which, in turn, cause changes in charge distribution and chemical properties that can be employed to improve desirable functional and nutritional properties of proteins.

Therapeutic Uses and Detoxification. SH-containing amino acids are used therapeutically to treat several diseases. For example, penicillamine [$HSC(CH_3)_2CH(NH_2)COOH$] is used to increase excretion of copper by patients suffering from Wilson's disease (Friedman, 1977b; Nielsen, 1989). This disease is caused by an inherited error in copper metabolism and is characterized by an accumulation of copper in the body. Schizophrenic patients also suffer from hypercupremia. Penicillamine is also used to treat rheumatoid diseases (Feldkamp, 1985), and as an antidote in cobalt, lead, mercury, and selenium poisoning (Baker and Czarnecki-Maulden, 1987; Friedman et al., 1973b; Henkin, 1974). Related aspects are described by Johnson (1991), Lowry and Baker (1989), Masri and Friedman (1974), and Singhall et al. (1987).

The chemical basis for these treatments is that penicillamine and related compounds form stable soluble chelates with metal ions which are removed from the circulatory system by urinary excretion. The transformations shown in Schemes 1 and 2 may also be involved in the action of penicillamine.

N-Acetyl-L-cysteine [$HSCH_2CH(NHCOCH_3)COOH$] has the ability to liquefy mucus and other secretions associated with pulmonary disorders (Patterson et al., 1985; *Physicians Desk Reference*, 1993). The compound is also reported to inhibit the virus causing AIDS and to stimulate T2 cells of the immune system (Droege, 1993). The mucolytic potency of the cysteine derivative and its possible use to treat viral diseases is presumably due to its ability to initiate sulfhydryl–disulfide interchange in susceptible proteins, leading to depolymerization and inactivation of active proteins.

The sulfhydryl–disulfide interchange, outlined earlier for protease inhibitors, merits further exploitation to inactivate other toxic proteins. These include ricin present in castor beans (Oda and Funatsu, 1977; Melby et al., 1993) and neurotoxins produced by pathogenic bacteria such as *S. typhimurium, Staphylococcus aureus, Listeria monocytogenes,* and *Enterobacterium coli* (Gill, 1979). These plant and bacterial proteins could be made harmless by modifying their three-dimensional structures through rearrangement of disulfide bonds. The practical feasibility of this approach, both in the food chain and for therapy of infected individuals, merits detailed study.

Related aspects include the use of sulfhydryl compounds as an antidote for acrylonitrile intoxication (Buchter et al., 1984), to trap nitrite (Byler et al., 1983; Lathia and Edeler, 1989), to alter lipid peroxides and lipoproteins (Gardner et al., 1985; Gavish and Breslow, 1991; Mercurio and Combs, 1987), to treat alcohol- and acetaminophen-induced liver and stomach injuries (Lindquist et al., 1991; Loguercio et al., 1993; Nelson, 1990), to prevent liver necrosis induced by the tricyclic antidepressant amineptine (Geneve, 1987), to reduce cisplatin nephrotoxicity (Appenroth and Winnefeld, 1993), to inactivate herbicides (Jablonkai and Hatzios, 1993; Venzil et al., 1990), to inhibit oral tumor formation (Trickler et al., 1993), and to protect against 5-fluorouracil-induced teratogenicity (Naya et al., 1987). However, sulfur amino acids failed to reduce mushroom (Schneider et al., 1992) and glucosinolate toxicities (Smith and Bray, 1992) and solasodine-induced liver enlargement in mice (Friedman, 1992b; unpublished results).

These studies reveal the following overlapping mechanisms by which SH-containing amino acids and peptides may exert their antitoxic effects *in vivo:* They may (a) prevent activation of inactive compounds to biologically active ones as described earlier for aflatoxin B_1; (b) serve as a source of glutathione, facilitating the actions of glutathione *S*-transferase, which catalyzes the conjugation of glutathione to the toxicants, forming soluble and inactive derivatives that are more readily eliminated by the kidneys than the parent compounds; (c) induce the formation of phase 2 enzymes such as quinone reductase which catalyze the transformation of toxic compounds to inactive forms; and (d) act as free-radical traps, whereby the free electrons of highly reactive hydroxyl, nitroxyl, and other radicals are transferred onto sulfur, where their reactivity is reduced because the electrons are dissipated through d-orbitals of sulfur atoms.

Glutathione–glutathione *S*-transferase interrelationships important in both detoxification and toxification of xenobiotics are described by Beutler (1989), Henning et al. (1991), Jones (1992), and Smith (1991).

CONCLUSIONS

Published studies reveal a complex interplay among nutrients, nonnutritive food ingredients, drugs, and other xenobiotics, and the health and welfare of animals and humans. At least three general approaches can be used to ameliorate adverse effects of the diet: (a) suppression of the biosynthesis of toxicants in plants; (b) inactivation or removal of toxicants from the food chain after harvest; and (c) alteration of the diet to counteract toxicant activity *in vivo*. My objective in this review is to integrate some of our findings and published information to develop a better understanding of the multidisciplinary interrelationships that influence food safety.

Sulfur compounds possess unique properties that benefit food safety, nutrition, and health. These include the primary role of disulfide bonds in governing three-dimensional structures of proteins and the ability of SH compounds to conjugate and detoxify xenobiotics and to quench potentially toxic free radicals. Beneficial effects of sulfhydryl compounds are also derived from metal chelation, whereby sulfur ligands sequester peroxidant Cu^{2+} and Fe^{2+} and potentially toxic As^{3+}, Cd^{2+}, Co^{3+}, Hg^{2+}, Pb^{2+}, and Se^{2+}, in both inorganic and organic compounds.

This limited overview shows that modifying sites of potentially toxic food ingredients should prevent them from interacting with living cells, especially susceptible cell receptor sites. Such modifications should contribute to our understanding of the basic mechanisms of toxicity and its prevention at the molecular level. They also demonstrate that such an approach is feasible with several classes of antinutrients and food toxicants and provide experimental evidence to facilitate formulating nutritionally optimal and safe food products. We are challenged to use the described fundamental molecular concepts, which look at food ingredients as organic compounds that are susceptible to predictable chemical and biochemical transformations, to develop better and safer foods and feeds (Friedman, 1994).

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