Nutritional Value and Safety of Heated Amino Acid–Sodium Ascorbate Mixtures†

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Browning reactions of amino acids and proteins with carbohydrates including vitamin C cause damage of food during processing and storage. To advance our knowledge of nutritional and toxicological consequences of browning, mice were fed for 14 days a nutritionally adequate casein diet supplemented at the expense of starch–dextrose with 5% of a series of amino acids previously heated in the dry state with sodium ascorbate (1:4 molar ratio). Growth inhibition by the heated mixtures ranged from none for arginine to significant for tryptophan. Additional studies revealed that (a) L-tryptophan–ascorbate significantly decreased weight gain when heated with an oven temperature of 200 or 215 °C but not when heated at 180 °C, (b) growth inhibition was less with *N*-acetyl-L-tryptophan–sodium ascorbate than with the tryptophan mixture, and (c) heated tryptophan or sodium ascorbate alone or tryptophan heated with glucose or ascorbic acid did not affect weight gain. These results complement previously reported studies with heated food protein–sodium ascorbate and ascorbic acid mixtures and suggest that tryptophan interacts with sodium ascorbate forming growth inhibitors. The possible chemical basis for the growth inhibition and its significance for food safety are discussed.

INTRODUCTION

Sodium ascorbate is widely used in many food applications; for example, the vitamin is added to flour before baking to improve bread dough characteristics and to bacon to prevent nitrosamine formation (Bauernfeind, 1982; Newark and Osdaca, 1974). In a previous paper (Friedman et al., 1987), we showed that sodium ascorbate (but not ascorbic acid) heated with wheat gluten and other proteins under conditions of crust baking strongly inhibits the growth of mice when added to an otherwise nutritionally adequate diet. The extent of inhibition depended on the temperature of heating in the range 180–215 °C and on sodium ascorbate concentration in the range 1–20%. Supplementation of the basal diet with sodium ascorbate heated in the absence of proteins had no effect on growth.

Elucidation of the nature and potency of the antinutritional material formed during heating of food proteins with sodium ascorbate is needed (a) to reveal the extent for which concern for food safety is warranted and (b) to develop food-processing conditions to prevent the formation of toxic material. Such work will lead to a better understanding of the fate of vitamin C in processed foods and to the prediction, discovery, and prevention of related adverse nutrient interactions produced by food processing.

The objective of this study was to establish whether consumption of various heated amino acid–sodium ascorbate mixtures also induces growth depression in mice.

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MATERIALS AND METHODS

Materials. L-Alanine, L-arginine hydrochloride, L-lysine hydrochloride, L-methionine, L-proline, L-serine, L-threonine, L-tryptophan, casein (ANRC), and AIN mineral mixture were obtained from U.S. Biochemical Corp., Cleveland, OH. *N*-Acetyl-L-tryptophan, L-cystine, L-glutamic acid, and L-glutamine were from Sigma Chemical Co., St. Louis, MO. Anhydrous β-D-glucose, L-ascorbic acid, sodium L-ascorbate, as well as the ingredients of the basal diet (Table I) except casein and the mineral mixture, were from ICN Nutritional Biochemicals, Cleveland, OH.

Oven Heating. A variety of amino acids mixed with sodium ascorbate in a molar ratio of 1:4 were heated in an oven at 180, 200, and 215 °C. Dry heating was performed in a Fisher Isotemp thermostat force-draft convection oven at temperatures of 180, 200, or 215 °C. Substances to be heated were ground together in a mortar. Approximately 100 g of the mixture was spread out on an enamel-coated tray to a thickness of approximately 6–8 mm. The tray was placed in the center of the oven cavity and heated for 72 min. The oven temperature was recorded with an Omega glass-braid insulated, 0.02-mm diameter, exposed-junction iron–constantan thermocouple. An identical thermocouple was inserted into the mixture and immobilized with clamps to the tray wall, so that the temperature of the reaction mixture was recorded as well.

Nutritional Studies. Nutritional studies were performed with weanling male mice (Swiss-Webster strain, initial weight approximately 9–12 g). The mice were kept two in each cage with free access to water and housed in a room with controlled atmosphere (relative humidity 60 ± 10%) and temperature (70 ± 2 °F). The oven-heated materials were mixed into a nutritionally balanced diet (Table I, control diet) at the expense of starch–dextrose. Five percent of the starch–dextrose portion of the casein control diet (Table I) was replaced by the heated mixture, and the modified diets were fed to mice for 14 days. Each mixture was fed to six mice ad libitum. Growth and feed intake were monitored regularly during the 14 days of the study.

Statistics. The data from the mouse bioassays were subjected to analysis of variance and to Duncan's test (Duncan, 1975). Heterogeneity of variance was not significant at $P > 0.05$. Standard errors of the mean (SEM) are listed in Tables II and III.

Table I. Composition of Basal Diet

ingredient[a]	amount, %
protein (casein)	10.00
Alphacel[b]	3.00
corn oil	8.00
cornstarch	27.36
dextrose	38.33
salts USP XIV[c]	5.00
sodium acetate	1.31
water (added)	5.00
complete vitamin mixture[d]	2.00
total	100.00

[a] The concentrations of the ingredients were not changed except for cornstarch–dextrose, which was adjusted to bring the total to 100%. [b] ICN Nutritional Biochemicals Corp., Cleveland, OH. [c] Supplemented with 125 mg/kg zinc and 5.7 mg/kg cobalt per kilogram of salt mixture. [d] Vitamin mixture provided in diet: vitamin A acetate, 18 IU/g. Cholecalciferol, 2.0 IU/g, and DL-α-tocopherol acetate, 0.11 IU/g, per kilogram of diet in milligrams (except as noted): choline chloride, 2.0 g; menadione, 20; nicotinic acid, 90; riboflavin, 20; pyridoxine hydrochloride, 20; thiamin hydrochloride, 20; calcium pantothenate, 60; D-biotin, 0.40; folic acid, 4.0; vitamin B_{12}, crystalline, 27 μg; inositol, 100; and ascorbic acid, 900.

Analysis for Sodium Ascorbate and Oxalic Acid. The sodium ascorbate and oxalate contents of heated mixtures were determined by standard methods (AOAC, 1980).

RESULTS AND DISCUSSION

The major objective of the current study was to determine if any of the heated mixtures would inhibit weight gain in mice, as was previously observed in analogous feeding studies with heated wheat gluten–sodium ascorbate mixtures (Friedman et al., 1987).

Figure 1 and Tables II and III show that growth inhibition did take place with most of the heated amino acid–sodium ascorbate mixtures, with the extent of inhibition dependent on the nature of the amino acid. The extent of inhibition in the first experiment listed in Table I was as follows: tryptophan > threonine or alanine > cystine > methionine > serine > tyrosine. No significant inhibition was noted with lysine or arginine. The second set of experiments in Table II shows that growth inhibition with proline (38%) was also significant; inhibition was not statistically significant with glutamic acid, glutamine, or tryptophan heated alone.

Since the two feeding experiments revealed that tryptophan heated with sodium ascorbate induced the strongest growth inhibition, additional experiments were carried out to further define the tryptophan effect. Table III shows that reducing the oven temperature from 215 to 200 °C did not change the growth-depressing effect of the sodium ascorbate–tryptophan mixture. Decrease in temperature to 180 °C, however, gave a heated mixture that sustained growth of mice approximately as well as the control diets; differences were not statistically significant. This temperature effect with tryptophan is similar to that observed with heated protein–sodium ascorbate mixtures (Friedman et al., 1987).

Substituting *N*-acetyl-L-tryptophan for L-tryptophan in the heating experiments at 215 °C resulted in a mixture that sustained growth better than L-tryptophan. A decrease in the temperature of heating to 180 °C, however, resulted in weight gains of 10.8 g with the L-tryptophan–ascorbate mixture and 8.0 g with *N*-acetyl-L-tryptophan–sodium ascorbate.

Substituting either ascorbic acid or glucose for sodium ascorbate before heating with L-tryptophan resulted in wight gains of 12.9 and 12.7 g, respectively, which did not differ statistically from the control value of 12.2 g. Thus, the growth-depressing effect is uniquely associated with sodium ascorbate.

Friedman et al. (1987) and Ziderman et al. (1989) have

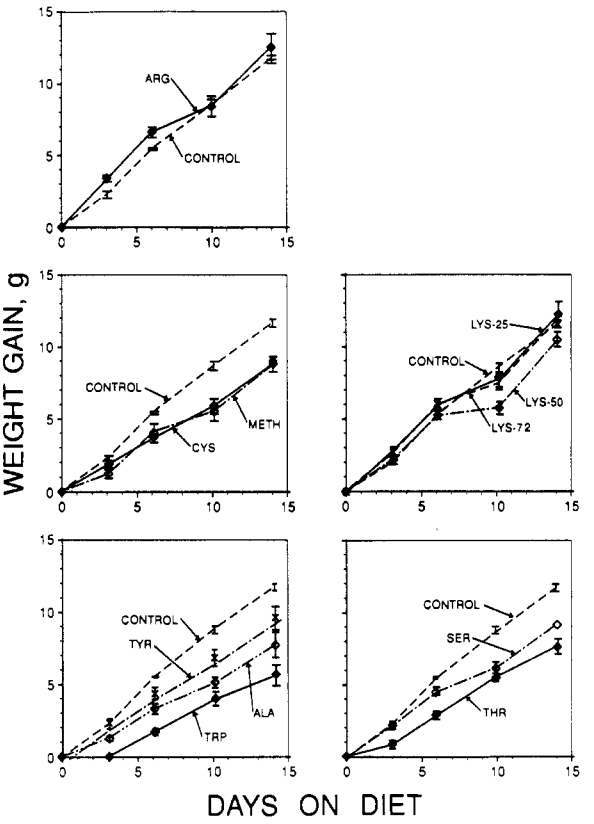

Figure 1. Effect of heated (215 °C/72 min) amino acid–sodium ascorbate mixtures (5% in diet) on weight gains in mice fed a standard casein diet for 4, 6, 10, and 14 days. Lys-25, Lys-50, and Lys-72 designate lysine–ascorbate mixtures heated for 25, 50, and 72 min, respectively.

Table II. Weight Gain in Mice Fed a Casein Diet for 14 Days Supplemented with Heated Amino Acid–Sodium Ascorbate Mixtures

sample[a]	body weight gain,[b] g	±SD	% of control
Experiment 1			
(1) tryptophan–sodium ascorbate	5.3[d]	1.6	47
(2) threonine–sodium ascorbate	7.2[c]	1.2	65
(3) alanine–sodium ascorbate	7.3[c]	1.9	65
(4) methionine–sodium ascorbate	8.4[bc]	1.2	75
(5) cystine–sodium ascorbate	8.5[bc]	0.5	76
(6) serine–sodium ascorbate	8.7[bc]	1.6	72
(7) tyrosine–sodium ascorbate	9.7[bc]	1.9	81
(8) lysine–sodium ascorbate	11.6[a]	1.0	104
(9) arginine–sodium ascorbate	12.0[a]	2.1	107
(10) control diet	11.2[a]	0.5	100
Experiment 2			
(1) proline–sodium ascorbate	7.6[c]	1.6	62
(2) glutamic acid–sodium ascorbate	10.4[b]	1.7	85
(3) glutamine–sodium ascorbate	10.5[ab]	1.8	86
(4) tryptophan	10.9[ab]	1.1	89
(5) control diet	12.3[a]	1.2	100

[a] The amino acid ascorbate in the mixture molar ratio was 1:4. The mixture was heated at 215 °C for 72 min. Starch–dextrose in the control diet (Table I) was replaced with the heated mixture to equal 5% of total diet. [b] Average weight gain of six mice ± standard deviation (SD). Means with no letter in common are significantly different, $P < 0.05$ (Duncan's multiple range test; Duncan, 1975). Pooled standard error of the mean (SEM) for each experiment ($n = 6$), ±0.6 g.

Table III. Weight Gain in Mice Fed a Casein Diet for 14 Days Supplemented with Tryptophan–Sodium Ascorbate and Related Materials

sample[a]	heating temp, °C	body weight gain,[b] g	±SD
L-tryptophan–sodium ascorbate	215	6.3[h]	1.7
N-acetyl-L-tryptophan–sodium ascorbate	215	9.9[def]	1.3
L-tryptophan–sodium ascorbate	200	6.9[gh]	2.0
N-acetyl-L-tryptophan–sodium ascorbate	200	8.4[fg]	2.5
L-tryptophan–sodium ascorbate	180	10.8[bcde]	1.6
N-acetyl-L-tryptophan–sodium ascorbate	180	8.0[fgh]	2.3
L-tryptophan–sodium ascorbate (1:1)	215	8.5[fg]	0.7
L-tryptophan–ascorbic acid	215	12.9[ab]	2.8
L-tryptophan–glucose	215	12.7[abc]	1.1
N-acetyl-L-tryptophan (1.25% in diet)	215	9.5[ef]	0.7
N-acetyl-L-tryptophan (1.25% in diet)	180	11.9[abcd]	1.7
N-acetyl-L-tryptophan (1.25% in diet)	unheated	12.6[abc]	0.8
L-tryptophan (1% in diet)	unheated	13.1[a]	1.5
control diet		12.2[abc]	1.4

[a] The L-tryptophan or *N*-acetyl-L-tryptophan to sodium ascorbate, ascorbic acid, or glucose in the heated mixture molar ratio was 1:4, unless otherwise indicated. Heating was for 72 min at the indicated temperatures. Starch dextrose (5%) of the control diet was replaced by the sample except as noted. [b] Average weight of six mice ± standard deviation (SD). Means with no letter in common are significantly different, $P < 0.05$ (Duncan's multiple range test; Duncan, 1975). Pooled SEM ($n = 6$), ±0.7 g.

previously speculated about possible causes of the heated protein–ascorbate-induced growth inhibition. They concluded that neither oxalic acid formation nor effects of dietary pH were major factors. Instead, they suggested that the intense internal aerobic exothermic reactions observed when carbohydrates are heated with proteins at 215 °C (Ziderman and Friedman, 1985) may both induce the formation and destruction of antinutritional compounds, depending on the interacting species. The recorded maximum internal temperatures in our heated mixtures were, as in the case of the protein–ascorbate mixtures, much higher than the oven temperature (Table IV). Noteworthy are the internal temperatures of the sodium ascorbate–tryptophan mixtures: 293 °C when heated at 215 °C and 287 °C when heated at 200 °C. Both these samples produced considerable growth depression. In contrast, the sample heated at 180 °C produced no increase in the internal temperature and no growth depression. However, the increase in internal temperature may not be the only factor affecting the formation of growth inhibitors since tryptophan–ascorbic acid has a large increase in temperature but produces no decrease in body weight.

Friedman et al. (1987) also showed that feeding heated pure sodium ascorbate under conditions identical with those of this study did not retard growth. It should be noted, however, that the oxidized form of ascorbic acid, dehydroascorbic acid, readily reacts with amino acids to produce brown products (El Sayed and Ashry, 1982; Hayashi et al., 1983; Namiki et al., 1986). Additional studies are needed to determine whether sodium ascorbate is oxidized to dehydroascorbic acid under the dry heating conditions used here and whether the browning products derived from dehydroascorbic acid can induce growth inhibition. It is also noteworthy that, since *N*-acetyl-L-tryptophan–sodium ascorbate produced less growth retardation than L-tryptophan/sodium ascorbate at 200

Table IV. Relationship between Oven Temperature and Maximum Temperature inside the Reaction Mixture during Heating Measured with a Thermocouple

sample[a]	oven temp, °C	max internal temp inside reaction mixture,[b] °C
L-alanine–sodium ascorbate	215	261
L-arginine–sodium ascorbate	215	247
L-cystine–sodium ascorbate	215	264
L-lysine–sodium ascorbate	215	288
L-methionine–sodium ascorbate	215	303
L-serine–sodium ascorbate	215	256
L-threonine–sodium ascorbate	215	264
L-tryptophan–sodium ascorbate	215	293, 302[c]
L-tyrosine–sodium ascorbate	215	249
N-acetyl-L-tryptophan–sodium ascorbate	215	232
L-glutamic acid–sodium ascorbate	215	220
L-glutamine–sodium ascorbate	215	236
L-proline–sodium ascorbate	215	312
L-tryptophan	215	252
L-tryptophan–sodium ascorbate	200	287
N-acetyl-L-tryptophan–sodium ascorbate	200	202
L-tryptophan–sodium ascorbate	180	181
N-acetyl-L-tryptophan–sodium ascorbate	180	181
L-tryptophan–sodium ascorbate (1:1)	215	223
L-tryptophan–ascorbic acid	215	262
L-tryptophan–glucose	215	216
N-acetyl-L-tryptophan	215	212
N-acetyl-L-tryptophan	180	177

[a] The L-tryptophan or *N*-acetyl-L-trytophan to sodium ascorbate, ascorbic acid, or glucose molar ratio was 1:4, unless otherwise indicated. Heating was for 72 min at the indicated temperatures. [b] Measured with a thermocouple wire inserted into the reaction mixture. [c] Results from two separate experiments.

and 215 °C (Tables II and III), the amino group in L-tryptophan probably participates in the heat-induced formation of toxic compounds. The data also suggest that the indole ring of both may also be involved.

Why sodium ascorbate and not ascorbic acid gives rise to deleterious material remains to be clarified. The course of the amino-carbonyl browning reaction in aqueous solution is known to be strongly pH dependent (Hayase and Kato, 1986; Nyhammar and Pernemalm, 1985). However, it is difficult to see how the nature of the acidic or basic microenvironment in the solid-state heating used here could influence the nature of products formed. Tryptophan residues are generally less susceptible to classical Maillard browning than lysine side chains of proteins (Friedman and Cuq, 1988). It is striking, however, that lysine and arginine, which showed no growth depression after heating with sodium ascorbate, were both hydrochlorides rather than free amino acids. Additional studies are needed to establish whether the HCl part of the molecule affects stabilities and toxicities of the reaction products.

Compositional analysis of the heated amino acid–ascorbic mixtures (Table V) revealed that the sodium ascorbate was nearly all destroyed during heating. The destruction of ascorbate was accompanied by the formation of oxalic acid. Since the oxalic acid content for all the amino acids was about the same, ranging between 3% and 4%, this acid does not appear to be responsible for the growth inhibition.

A major factor that may be responsible for determining the nature and concentrations of deleterious compounds formed may be the sharp rise in internal temperature of the reaction mixture during heating in the dry state

Table V. Sodium Ascorbate and Oxalic Acid Content of Amino Acid–Sodium Ascorbate Mixtures

material[a]	sodium ascorbate in sample, %	oxalic acid in sample, %
sodium ascorbate plus		
L-alanine	<0.2	3.46
L-arginine	<0.2	3.10
L-cystine	<0.2	4.61
L-lysine	<0.2	3.01
L-methionine	<0.2	3.69
L-serine	<0.2	3.27
L-threonine	<0.2	3.68
L-tryptophan	<0.2	3.37
L-tyrosine	<0.93	3.67

[a] Amino acid–sodium ascorbate mixtures (molar ratio 1:4) were heated at 215 °C for 72 min.

measured by a thermocouple wire inserted directly into the blend during the heating process. This exothermic effect may also contribute to the observed formation of mutagenic compounds during heating of protein–carbohydrate including ascorbate mixtures (Friedman et al., 1990). As discussed elsewhere (Friedman et al., 1987, 1989), the pH of the reaction mixture or of the diet is probably not responsible for the observed inhibition.

CONCLUSIONS

In conclusion, the present results show that the earlier observation that sodium ascorbate heated with proteins results in the formation of antinutritional compounds also applies to amino acids, especially tryptophan. The reduction in weight gain of mice fed a nutritionally adequate diet supplemented with these materials suggests that heating induces the formation of nutritionally antagonistic or toxic compounds that interfere with essential metabolic pathways such as digestion, transport, absorption, and utilization of nutrients (Gumbmann et al., 1983; Oste and Sjodin, 1984; Friedman, 1989). Further studies of the chemical basis of these effects may be more conveniently performed with tryptophan or other amino acid–ascorbate mixtures than with the more complex protein–ascorbate blends since the heat-induced products may be easier to isolate and characterize.

The amounts of sodium ascorbate used in thermal food processing to improve bread-dough characteristics such as loaf volume and bread texture (Bauernfeind, 1982; Ashoor and Monte, 1984) and to inhibit nitrosamine formation in bacon (Newark and Osadca, 1974; Izumi et al., 1985) are much less than in the model heating experiments used in this study. Our experiments were designed to approximately mimic the ratios (but not concentrations) of sodium ascorbate to lysine content of real foods. Since our findings do not rule out possible cumulative biological effects of any antinutritional or antimetabolic compounds formed during heat treatment, additional studies are needed to determine whether consumption of low levels of the heat-derived compounds may constitute a human health hazard.

These considerations suggest the need (a) to characterize the compound(s) in heated protein- and amino acid–sodium ascorbate mixtures that may be responsible for the observed growth inhibition; (b) to determine the safety of the pure compounds in laboratory animals and measure their prevalence in commercial foods to define possible human risk; (c) to carry out studies with related food ingredients such as sodium citrate, sodium gluconate, and sodium glutamate to define the mechanism of this type of growth inhibition; and (d) to devise processing conditions to prevent the formation of the growth inhibitors in food.

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