Ability of Amino Acids, Dipeptides, Polyamines, and Sulfhydryls To Quench Hexanal, a Saturated Aldehydic Lipid Oxidation Product

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Hexanal is a common product arising from the oxidation of $\omega-6$ fatty acids. Because aldehydic lipid oxidation products can react with food components, interactions between hexanal and sulfhydryl- and amine-containing compounds were determined. The polyamines, spermidine and spermine, and the sulfhydryl-containing compounds, glutathione and thioctic acid, decreased headspace hexanal concentrations ≤7.0%. Histidine was the only amino acid tested that was able to quench headspace hexanal. Histidine-containing dipeptides decreased headspace hexanal 3.0−8.5-fold more than histidine. Hexanal quenching by the hisitidine-containing dipeptides increased as the size of the aliphatic side group of the amino acid adjacent to histidine increased, with Leu-His having the greatest hexanal quenching activity. The ability of Leu-His to quench histidine increased with increasing pH. The ability of histidine-containing dipeptides to interact with hexanal suggests that it may be possible to design peptides to alter the concentration of saturated aldehydes in oxidizing lipids.

Keywords: *Dipeptides; polyamines; glutathione; carnosine; hexanal; lipid oxidation*

INTRODUCTION

Autoxidation of lipids in biological and food systems is accompanied by the formation of a variety of aldehydic compounds, including saturated, α,β-monounsaturated, polyunsaturated, and hydroxylated aldehydes (Poli et al., 1985; St. Angelo et al., 1987). These aldehydic compounds are capable of reacting with biomolecules causing various deleterious effects in foods (e.g., loss of protein functionality and discoloration of myoglobin; Xiong and Decker, 1995; Chan et al., 1997) and in biological tissues (e.g., oxidative modification of DNA, enzymes, and lipoproteins; Brooks and Klamerth, 1968; Reiss et al., 1972; Yau, 1979; St. Angelo et al., 1987; Szweda et al., 1993; Alaiz et al., 1994).

Hexanal and propanal are two major saturated aldehydes that originate from the breakdown of $\omega-6$ and $\omega-3$ fatty acid peroxides, respectively (Frankel, 1993; Yasuhara and Shibamoto, 1995). These two aldehydes are frequently detected during the oxidation of lipids in foods and biological systems (Ajuyah et al., 1993; Nawar, 1996). Saturated aldehydes are believed to be one of the main contributors of food rancidity (Frankel, 1982), possibly because they are less reactive than unsaturated aldehydes and thus their concentrations do not change dramatically in the presence of nucleophilic biomolecules, as does that of unsaturated aldehydes (Zhou and Decker, 1999). However, in foods and related biological tissues, there are a variety of compounds that could potentially react with saturated aldehydic compounds through processes such as Schiff base reactions including proteins, peptides, amino acids, polyamines, and sulfhydryls. Little is known about the ability of these compounds to interact with and thus alter the concentration of saturated aldehydes.

In a previous paper, the ability of carnosine and related compounds to quench various unsaturated aldehydes was reported (Zhou and Decker, 1999). The objective of this research was to investigate the ability of various amine and sulfhydryl compounds to react with the saturated aldehyde hexanal.

MATERIALS AND METHODS

Materials. Glutathione (reduced) and spermidine were purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI). Leucine, β-alanyl-β-alanine (β-Ala-β-Ala), leucylglycine (Leu-Gly), valylhistidine (Val-His), leucylhistidine (Leu-His), and isoleucylhistidine (isoLeu-His) were purchased from BACHEM Bioscience Inc. (King of Prussia, PA). Methylene chloride and acetonitrile (HPLC grade) were from Fisher Scientific (Fair Lawn, NJ). The following chemicals were obtained from Sigma Chemical Co. (St. Louis, MO): L-carnosine (>99%, lot 27H0883), homocarnosine, glycylhistidine (Gly-His), alanylhistidine (Ala-His), histidyl-leucine (His-Leu), dl-6,8-thioctic acid (reduced form), hexanal, spermine, L-alanine, β-alanine, L-valine, glycine, DL-lysine, γ-aminobutyric acid (GABA), and imidazole.

Interaction of Amine- and Sulfhydryl-Containing Compounds with Aldehydes. Amino acids and dipeptides were examined for contaminating hydrazine as described by Zhou et al. (1999). Hydrazine was only detected in carnosine, which was contaminated at a level of 0.01% (wt). Hydrazine was removed from carnosine by following the procedure of Zhou and Decker (1999). Amine- and sulfhydryl-containing compounds (0.1−10 mM) in 0.12 M KCl/5 mM phosphate buffer (pH 7.4 and 8.5) were mixed with hexanal (0.5 mM), sealed in headspace vials preflushed with nitrogen, and incubated at 40 °C in the carousel of a Hewlett-Packard 19395A headspace sampler (Avondale, PA) for up to 5 h. Headspace hexanal concentration was analyzed by gas−liquid chromatography (GLC) (Zhou and Decker, 1999).

Measurement of Leucylhistidine, Hexanal, and Their Reaction Products. Leu-His (5 mM) and hexanal (0.5 mM)

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Table 1. Decrease in Headspace Hexanal Concentration after 1 h of Incubation of Polyamine and Sulfhydryl Compounds with Hexanal (0.5 mM) in 0.12 M KCl/5 mM Phosphate Buffer (pH 7.4)[a]

compound	polyamine/sufhydryl concn (mM)	remaining headspace hexanal (%)
spermine	5.0	95.2 ± 0.5**
spermidine	5.0	96.2 ± 0.9**
glutathione	0.1	100.2 ± 0.3
	0.5	100.0 ± 0.5
	1.0	98.2 ± 0.2**
	5.0	92.6 ± 0.5**
thioctic acid	0.1	99.5 ± 0.1
	0.5	98.7 ± 0.4
	1.0	99.6 ± 0.7
	5.0	96.2 ± 1.0**

[a] Data represent means ± standard deviation of triplicate analyses. * and ** indicate that headspace hexanal concentrations were significantly reduced compared to controls at the levels of $p \leq 0.05$ and 0.01, respectively.

in 0.12 M KCl/5 mM phosphate buffer (pH 7.4) were incubated at 40 °C, and changes in their concentrations were analyzed by HPLC and GLC, respectively, after 1, 3, and 5 h from separate vials. The HPLC system consisted of a Waters model 510 pump, a Waters 740 integrator (Milford, MA), and a Hitachi L-4200 UV−vis detector operating at 210 nm (Hitachi Instruments, Inc.). Separation of Leu-His was achieved using a Hypersil ODS (C18) column (5 μm; 4.6 × 250 mm; Alltech Associates Inc., Deerfield, IL). The mobile phase ran at 1.5 mL/min and consisted of 0.1 M phosphate buffer (pH 7.4) and methanol (50:50, v/v). The hexanal in phosphate buffer (1 mL) was extracted with methylene chloride (2 mL) containing *n*-octane as an internal standard. The extracted hexanal in methylene chloride was analyzed by GLC (Zhou and Decker, 1999), and its concentration was calculated according to the FID response factor, peak area, and *n*-octane concentration.

Measurement of Reaction Constant between Leucylhistidine and Hexanal. Kinetic studies were performed by incubating 0.5 mM of hexanal with an excess amount of Leu-His (10 mM) in 0.12 M KCl/5 mM phosphate buffer (pH 5.0−8.0) at 40 °C. The reaction mixture (1 mL) was sampled every 10 min for 60 min, and hexanal was analyzed by GLC as described above. The reaction rate constant was obtained by plotting reaction time (t) against $\ln(a/a_x)$, where a and a_x represent initial and time x concentrations of hexanal, respectively (Tinoco et al., 1985).

Statistical Analysis. All experiments were performed on triplicate samples and were repeated at least twice. Differences between means were determined using the Student t test using Minitab release 7.2 software.

RESULTS

Spermine (5 mM) and spermidine (5 mM), two polyamines found in foods, were capable of reducing 5 and 4% of headspace hexanal, respectively, after 1 h of incubation at pH 7.4 and 40 °C (Table 1). Reduced glutathione (5 mM) and reduced thioctic acid (5 mM), sulfhydryls present in foods, reduced 7 and 4% of headspace hexanal, respectively. Decreasing reduced glutathione and reduced thioctic acid to concentrations ≤1 mM resulted in little or no reduction in headspace hexanal.

Table 2 shows the ability of various amino acids and dipeptides (5 mM) to reduce headspace hexanal concentrations after 1 h of incubation with 0.5 mM hexanal at 40 °C and pH 7.4 or 8.5. All amino acids tested except for histidine had no effect in headspace hexanal concentration, suggesting that the α- (e.g., alanine), β- (e.g., β-alanine), and γ- (e.g., GABA) amino groups of amino acids do not readily interact with the carbonyl group of hexanal. The inability of lysine to decrease headspace hexanal concentration indicates that the ϵ-amino group also does not readily react with hexanal. Three non-histidine dipeptides with the free amino group in the α-position, Ala-Gly, Gly-Ala, and Leu-Gly, quenched headspace hexanal (4−5%), indicating that the peptide bond increased the reactivity of the α-amino group. The presence of a peptide bond has also been found to increase the antioxidant activity of carnosine compared to its constituent amino acids, β-alanine and histidine (Kohen et al., 1988). Similar activity observed between Ala-Gly and Gly-Ala suggests that the order of amino acids in non-histidine-based dipeptides had no influence on their quenching activity. β-Ala-β-Ala, a dipeptide with a free amino group in the β-position, was inert to hexanal, suggesting that the influence of the peptide bond could not be extended as far as the β-amino group.

Table 2. Decrease in Headspace Hexanal Concentrations after 1 h of Incubation of Various Dipeptides and Amino Acids (5 mM) with Hexanal (0.5 mM) in 0.12 M KCl/5 mM Phosphate Buffer at 40 °C and pH 7.4[a]

peptide or amino acid	remaining headspace hexanal (%) pH 7.4	pH 8.5
L-alanine	101.1 ± 0.3	
β-alanine	99.7 ± 1.3	
L-valine	99.4 ± 0.8	
glycine	102.0 ± 2.0	
leucine	100.5 ± 0.2	
γ-aminobutyric acid	101.4 ± 0.6	
DL-lysine	99.9 ± 1.0	
β-alanyl-β-alanine	100.0 ± 0.2	
alanylglycine	96.1 ± 5.1	
glycylalanine	96.3 ± 0.3**	
leucylglycine	94.9 ± 0.3**	
imidazole	100.7 ± 0.6	90.9 ± 0.5**
L-histidine	97.7 ± 0.9*	97.9 ± 1.0*
β-alanylhistidine (carnosine)	96.8 ± 0.4**	98.3 ± 0.1**
γ-aminobutyrylhistidine (homocarnosine)	100.1 ± 0.1	85.7 ± 0.5**
glycylhistidine	93.0 ± 0.2**	
alanylhisitine	91.6 ± 0.1**	79.7 ± 0.6**
valylhistidine	90.4 ± 1.5**	84.6 ± 0.1**
leucylhistidine	82.2 ± 0.7**	72.6 ± 1.3**
isoleucylhistidine	89.5 ± 0.3**	83.7 ± 0.7**
histidyl-leucine	80.3 ± 0.1**	76.5 ± 0.7**

[a] Data represent means ± standard deviations of triplicate analyses. * and ** indicate that headspace hexanal concentrations were significantly reduced compared to controls at the levels of $p \leq 0.05$ and 0.01, respectively.

Histidine was observed to have weak hexanal ($p \leq 0.05$) quenching activity (Table 2; 2% reduction in headspace concentration) at pH 7.4. Histidine-based dipeptides with free amino groups in the α-position (e.g., Ala-His and Leu-His) exhibited improved hexanal quenching activity (7.0−19.7% reduction). Carnosine and homocarnosine, with their free amino group in the β-position (β-alanine) or in the γ-position (GABA), respectively, had hexanal quenching activity similar to or lower than that of histidine alone. His-Leu and Leu-His were the most effective dipeptides tested, reducing 20 and 18% headspace hexanal, respectively, after 1 h of incubation. The similarity in hexanal quenching activity between these two dipeptides further supports the fact that the order of amino acids has little influence on their reactivity. Increase in the size of the hydrophobic alkyl group increased hexanal quenching activity (e.g., Leu-His>Val-His > Ala-His > Gly-His). iso-Leu-

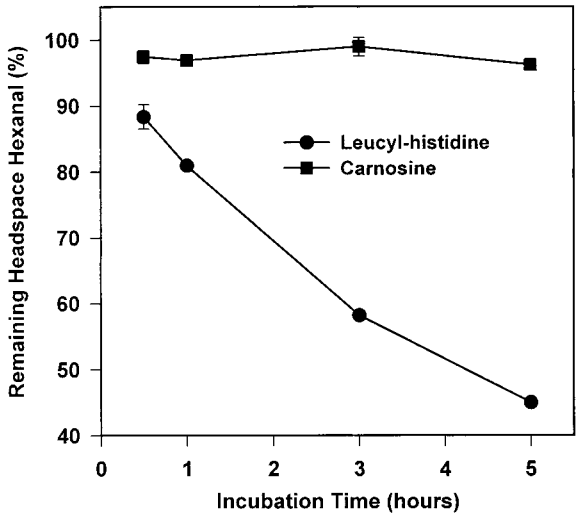

Figure 1. Decrease in headspace hexanal concentration (0.5 mM) during 5 h of incubation with carnosine (5 mM) and leucylhistidine (5 mM) at 40 °C and pH 7.4.

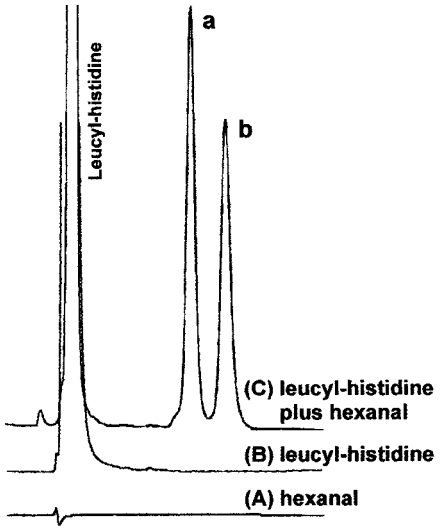

Figure 2. High-performance liquid chromatograms of (A) hexanal (4.6 mM), (B) leucylhistidine (5 mM), and (C) their mixture. The mixture was incubated for 1 h at 40 °C and pH 7.4.

His had lower activity than Leu-His, indicating that a branched methyl group at the first carbon of the side chain had less impact on hexanal quenching activity than a branched methyl group at the second carbon of the side chain. Increase in pH from 7.4 to 8.5 increased the quenching ability of the histidine-based dipeptides with a free amino group at the α-position (Table 2). No changes were observed for carnosine and homocarnosine when the pH was increased from 7.4 to 8.5. Figure 1 shows changes in headspace concentrations of hexanal (0.5 mM) during 5 h of incubation with carnosine and Leu-His (5 mM). Leu-His reduced 42 and 55% of headspace hexanal after 3 and 5 h of incubation, respectively, whereas carnosine led to only a 3% reduction under the same conditions.

To confirm that changes in headspace aldehyde concentration reflect chemical and not physical effects, HPLC was used to monitor Leu-His concentrations during incubation with hexanal. Figure 2 shows the HPLC chromatograms of hexanal (A), Leu-His (B), and their mixture (C) after 1 h of incubation at 40 °C and pH 7.4. At least two different reaction products were

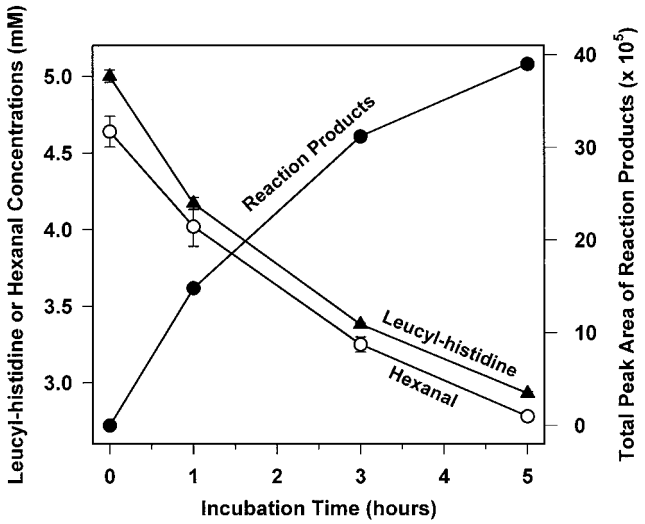

Figure 3. Decrease in concentrations of leucylhistidine and hexanal and increase in total peak area of the reaction products of leucylhistidine (5 mM) plus hexanal (4.6 mM) during 5 h of incubation and pH 7.4. Hexanal concentrations were determined by using *n*-octane as an internal standard.

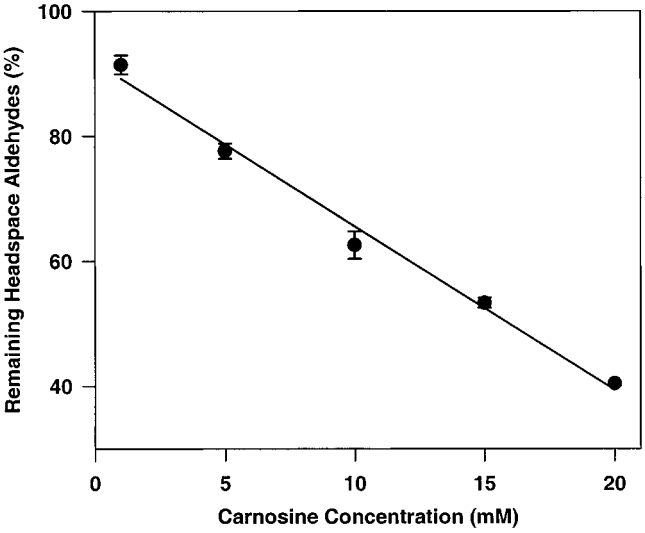

Figure 4. Effect of different concentrations of Leu-His (1−20 mM) on the quenching of headspace hexenal. Leu-His was incubated with hexanal (0.5 mM) in 0.12 M KCl/5 mM phosphate buffer (pH 7.4) at 40 °C for 30 min.

detected in the mixture of Leu-His plus hexanal. The different reaction products observed by HPLC could represent the formation of adducts at different sites (e.g., imidazole and α-amine groups). The decrease in Leu-His concentration was well correlated with the decrease in hexanal concentration and the increase in total peak area of the reaction products (Figure 3).

Kinetic studies showed that the reaction between Leu-His and hexanal is second-order overall and first-order with respect to each of the reactants. This was supported by the following observations: (1) At 0.5 mM of hexanal concentration and pH 7.4, increasing Leu-His concentration resulted in a linear decrease in headspace hexanal concentrations (Figure 4); (2) At fixed Leu-His concentration of 5 mM and pH 5−8, straight lines were obtained when reaction time (t) was plotted against ln-(a/a_x), where a and a_x represent concentrations of hexanal at the beginning of or during the course of reaction, respectively (Figure 5). Reaction rate constants between Leu-His and hexanal were obtained at 40 °C

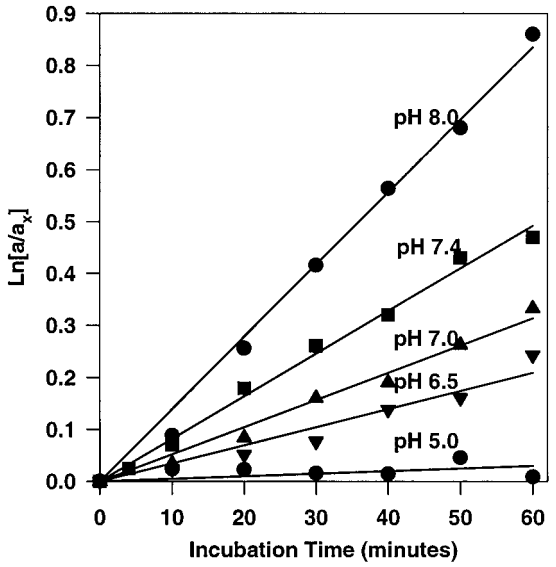

Figure 5. Plot of reaction time against ln(a/a_x), where a and a_x represent initial and time x concentrations of hexanal, respectively.

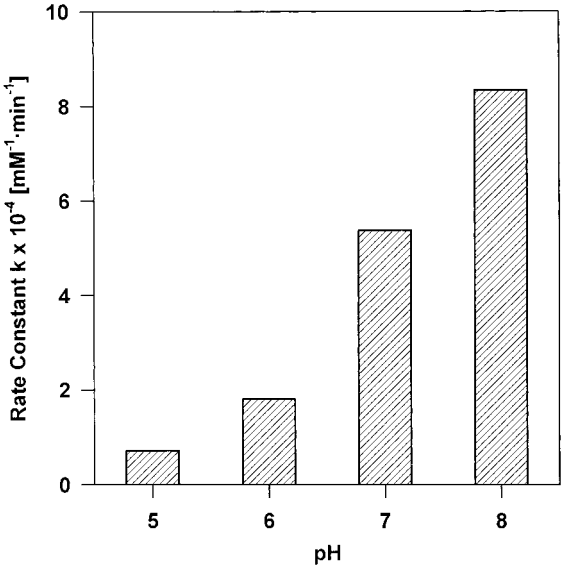

Figure 6. Effects of pH (5.0−8.0) on the reaction between Leu-His and hexanal. Leu-His (10 mM) was incubated in 0.12 M KCl/5 mM phosphate buffer with hexanal (0.5 mM) at 40 °C. The hexanal concentrations were determined by using *n*-octane as an internal standard.

and pH from 5 to 8 (Figure 6). Increase in pH resulted in large increases in rate constants with the reactivity between Leu-His and hexanal at pH 8 being 12 times higher than that at pH 5. This is understandable because nucleophilic reactions such as the Schiff base and Michael addition reactions increase with increasing pH (Solomons, 1984).

DISCUSSION

Foods contain various compounds that can potentially interact with aldehydes including amino acids, peptides, polyamines, and sulfhydryls. Carnosine and related dipeptides, sulfhydryls, and polyamines have been found to be effective in quenching α,β-unsaturated aldehydes including 4-hydroxyl-2-*trans*-nonenal (Zhou and Decker, 1999). However, the above data show that these compounds were less effective and sometimes ineffective in quenching saturated aldehydes. The exception to this were histidine-containing dipeptides that contained an amino acid with an amino group at the α-position. However, such dipeptides are not normally present in foods and biological systems at high concentrations. Therefore, the ability of sulfhydryls and amines common to foods to quench unsaturated but not saturated aldehydes could explain why saturated aldehydes are the better marker of lipid oxidation in biological systems such as meat (Konopka et al., 1995).

Saturated aldehydes arising from the breakdown of lipid peroxides are one of the major contributors of rancidity in lipid-containing food samples. Histidine-based dipeptides with the free amino group in the α-position are able to quench hexanal under the conditions used in this study. The presence of two reaction products (Figure 2) from the interaction of hexanal with Leu-His suggest that the carbonyl group of hexanal is reacting with both the free α-amino group and the imidazole nitrogens. The increased reactivity of dipeptides compared to amino acids is likely due to increased reactivity of the α-amino group via the effect of the peptide bond (as seen with increased reactivity of non-histidine-based dipeptides), increased reactivity of the imidazole nitrogens, and/or changes in stereochemistry that alter reactivity. The ability of histidine-containing dipeptides to quench aldehydes was enhanced by amino acids with large aliphatic side groups. Further studies are necessary to identify the Leu-His−hexanal products to better understand the mechanisms of dipeptide-saturated aldehyde interactions. Understanding these mechanisms may lead to the design of novel dipeptides to suppress lipid oxidation derived off-flavors in foods.

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