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DIETARY D-AMINO ACIDS

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INTRODUCTION

Foods contain a large assortment of xenobiotics (foreign, unnatural substances) that can have both positive and negative nutritional implications (32). One example is the occurrence of the uncommon D-stereoisomers of amino acids in some dietary proteins. These D-amino acids are produced from the common L-stereoisomers during food preparation and processing. Their presence decreases the digestibility of dietary protein and the availability of its component amino acids. Although D-amino acids have generally been considered to have a deleterious effect on nutrition, accumulating evidence suggests that in some special instances D-amino acids may be beneficial.

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In the mid-19th century, Pasteur demonstrated that aspartic acid derived from vetch plants was optically active (chiral), whereas aspartic acid synthetically produced by heating ammonium fumarate was optically inactive (66). It was subsequently determined that only L-amino acids are present in the proteins of living organisms, even though the D- and L-stereoisomers (enantiomers) have identical chemical and physical properties except for rotating plane polarized light in opposite but equal directions. Organisms have L-amino acids in their proteins because of the stereospecificity of biological protein synthesis (79). The origin of this stereoselectivity is not resolved even though the problem has been studied for over a century (5).

With the advent of routine analytical methodologies for separating amino acid enantiomers, D-amino acids were found to occur in some organisms. For example, the peptidoglycans of bacterial cell walls contain D-aspartate, D-glutamate, and D-alanine (4, 70). Some insects and marine invertebrates have D-amino acids as major components of their cellular fluids (21, 24, 28, 60). In certain marine bivalves, D-amino acids make up 1% of the wet weight of the organism (29). Higher plants also contain D-amino acids (71). Metabolically stable proteins in long-lived mammals accumulate D-aspartic acid with age generated from the L-isomer via in vivo racemization (2). D-Aspartic acid has been found in concentrations of about 3% [% = (D/D+L)100] in the white matter of human brain (58), and as high as 10% in purified myelin basic protein (33). Evidence suggests that aspartic acid undergoes in vivo racemization in all human tissues (20), although it does not generally accumulate in detectable quantities because of the rapid turnover rates of most proteins (2).

Chiral amino acids can be converted into a racemic mixture (e.g. one in which the ratio of D- to L-amino acids is 1.0) via the racemization reaction shown below (3):

$$\text{L-amino acid} \underset{k_i}{\overset{k_i}{\rightleftarrows}} \text{D-amino acid.} \qquad 1.$$

The mechanism of the reaction involves the abstraction of the α-hydrogen of the amino acid, generating a planar carbanion intermediate. Racemization follows the rate expression:

$$\ln \left\{ \frac{1 + \text{D/L}}{1 - \text{D/L}} \right\} = 2\, k_i t. \qquad 2.$$

The rate of racemization ($2k_i$) depends on whether the amino acids are free or peptide bound and is a function of temperature and pH. The R-substituent is

the principal agent that determines the relative racemization rates of the individual amino acids (3). Representative racemization half-lives for free and peptide-bound serine, aspartic acid, alanine, and isoleucine are given in Table 1. These amino acids were chosen because they represent the range of racemization rates of the protein amino acids.

Racemization of serine, cysteine, and threonine generate not only the corresponding D-enantiomers, but in addition some xenobiotic amino acids. For example, the carbanion intermediate of serine rapidly eliminates OH^-, yielding dehydroalanine, an amino acid that can react with a variety of nucleophiles (34, 57, 59). The reaction of dehydroalanine with the ϵ-NH_2 group of lysine yields lysinoalanine [N^{ϵ}-(D,L-2-amino-2-carboxyethyl)-L-lysine], an amino acid in which the alanine moiety is racemic whereas the lysine component is chiral. In dietary proteins, this reaction results in cross-linking, which decreases digestibility (19, 38). Dietary lysinoalanine also has toxic properties (49).

From a nutritional standpoint, racemization of the essential amino acids is of central importance. Utilization and metabolism of the D-enantiomers of the essential amino acids have been investigated since the beginning of this century. These early studies, which were extensively reviewed by Neuberger (64) and Berg (8), demonstrated that in mammals the D-stereoisomers of the essential amino acids were poorly utilized, in some cases caused growth inhibition, and were mainly excreted in the urine. Recent investigations have confirmed these findings (35, 36, 52, 75). Only within the last few years have racemization rates of essential amino acids been investigated. The available

Table 1 Amino acid racemization half-lives for the listed amino acids exposed to various conditions[a]

	Half-life			
	Serine	Aspartic acid	Alanine	Isoleucine
100°C, pH 7–8				
Free amino acids (3, 78)	3 days	30 days	120 days	300 days
Poly amino acids (78)	10 hr	22 hr		
Aspartame (Asp-Phe-oMe) (10)		13 hr		
Amino acid esters 20°C, pH 7, pyridoxal 5'-phosphate[b] (69)		4 min	4 min	7 min
Casein 83°C, pH 9 (56)	16 hr	19 hr	11 days	57 days
Soybean protein, 75°C, 0.1-N NaOH (37)	<9 min	<20 min	5 hr	25 hr
Chicken muscle 121°C, 15% water (55)	4 days	5 hr	11 days	18 days

[a] One half-life is the time required to reach a D/L ratio of 0.33 and equals $\ln 2/2k_i$, where k_i is the rate of interconversion of amino acid enantiomers (see Equations 1 and 2).

[b] The ratio of the pyridoxal 5'-phosphate to amino acid concentrations is 1.0.

data are rather meager, and only approximate rates are available. Racemization half-lives for the essential amino acids in humans (6) are given in Table 2. Cysteine is especially prone to racemization, whereas amino acids with aliphatic side chains are the most stable with respect to racemization. Most essential amino acids have racemization half-lives longer than that of aspartic acid (see Table 1).

Food preparation in which proteins are exposed to alkali and/or high temperatures for long periods may generate appreciable amounts of D-amino acids from racemization. Dakin was one of the first to demonstrate that dietary proteins exposed to heat and strong alkali had reduced digestibilities (22, 23). It is now apparent that digestibility decreases dramatically with increasing lysinoalanine content and racemization of amino acid residues (14, 19, 38, 40, 47, 48, 57).

The study of dietary D-amino acids produced primarily from racemization has become an active area of research during the last decade. This review presents some of the results obtained in these investigations and their nutritional implications.

DIETARY SOURCES

Free D-amino acids probably do not constitute a major dietary component. Although some insects, worms, and marine invertebrates contain significant

Table 2 Racemization half-lives of the essential amino acids for humans[a]

	100°C pH 7–8 (days)	0.1-N NaOH 75°C (hr)[g]
Isoleucine, leucine, valine	300[b]	24
Phenylalanine, tyrosine	50[b]	2–6
Lysine	40[c]	8
Tryptophan	40[d]	
Threonine	20[e]	2
Methionine	30[f]	3
Cysteine	2[e]	<30 min

[a] Tyrosine and cysteine are considered semiessential since they can be synthesized from phenylalanine and methionine, respectively.

[b] Based on inductive effects, the individually grouped amino acids should have similar racemization rates (3). The half-life for isoleucine was used as a representative value for those with aliphatic side chains (see Table 1). The value listed for the aromatics is for phenylalanine (1).

[c] Taken from Engel & Hare (27).

[d] The rate for tryptophan was estimated to be similar to that of lysine based on measurements at pH 9, 83°C (56).

[e] Based on measurements at pH 9, 83°C (56) and calculated relative to the alanine $t_{1/2}$ of 120 days (see Table 1).

[f] From Boehm & Bada (11).

[g] Soybean protein (37).

quantities of free D-amino acids, these organisms are generally not major dietary components of the overall human population. In societies where marine bivalves are an important dietary protein source, questions have been raised about the nutritional and toxic effects of ingesting large quantities of D-amino acids (29).

Many of the treatments used to modify proteinaceous food substances, including conditions employed in cooking, involve exposure to heat and alkaline conditions. This induces racemization, generating D-amino acids in the component proteins. It has been shown that significant amounts of D-amino acids are present in some processed and commercially available foods (40, 50, 55, 59). Lysinoalanine appears to be a ubiquitous dietary substance (57). In addition, manufactured products such as the synthetic dipeptide sweetener aspartame are especially prone to racemization under certain conditions (10).

Natural Products

Milk, meat, and grain proteins, which do not contain significant amounts of D-amino acids, are often exposed to conditions that could cause racemization while being prepared for consumption. Milk and its products may represent the best example of this effect during the processing of a natural product. Although "raw" (untreated) milk and milk products are available from health food stores, most milk products first undergo pasteurization (heating for 30 minues at 145–165°F) or ultrapasteurization (275–300°F for 15 seconds). Other processing steps include homogenization, condensation, "velvetizing," and the production of specialized products such as buttermilk, yogurt, and cheese from various milk fractions. In the case of these latter products, milk is fermented with bacteria, which could be a biological source of D-amino acids.

In order to investigate the extent of racemization during the treatments that raw milk and its products undergo, the D-aspartate content of a series of processed milk samples and milk products was determined. These results are shown in Table 3. Untreated raw milk has the lowest D-aspartate content. There is an accumulation of D-aspartate with increasing extent of processing. Products that require heating, such as condensed or evaporated milk, show D-aspartate contents over 3%. In the highest range are casein and soy-based infant formulas, which undergo processing steps involving spray-drying and sterilization with heat.

Processed Foods

Modern food technology utilizes a variety of processes to modify natural proteins in order to improve taste, texture, and shelf life. Primary among these are heat and alkali treatments used to produce protein products with special properties, shapes, or functions. Soy protein, for example, is treated with alkali and heat during extrusion to produce textured fibers used in meat

Table 3 D-Aspartic acid content of milk, processed milk products, and milk substitutes (R. Chemburkar and E. H. Man, unpublished results)[a]

Product	% D-Aspartic acid[b]
Unprocessed (raw) milk	1.48
Acidophilus milk	2.05
Nonfat powdered milk	2.15
Buttermilk	2.44
Condensed milk	2.49
Yogurt (plain)	3.12
Evaporated milk	3.25
Vitamin D milk	3.36
Infant formula	
(milk base)	4.95
(soy base)	3.64
(soy base)[c]	10.80

[a] Samples were hydrolyzed in 6-N HCl at 100°C for 24 hr. Enantiomeric analyses were carried out as described elsewhere (67).

[b] % D-Asp = (D/D+L)100; 50% D-amino acid = a D/L ratio of 1.0, or complete racemization.

[c] Friedman et al (38).

analogs and extenders. Corn protein is processed with alkali in the form of aqueous lime, ashes, or caustic soda to produce products such as corn chips or tortillas.

Table 4 contains data reported on the D-amino acid content of processed foods as compared with unprocessed controls. In all instances, heat, or the combination of heat and alkali, produced measurable quantities of D-amino acids. Casein that had been heated to 230°C (20 minutes) contained 31% D-aspartic acid, the highest value we have found. For the amino acids shown, the most highly racemized is aspartic acid. Some amino acids not listed, such as serine and cysteine, would probably be more extensively racemized than aspartic acid (see Tables 1 and 2). In general, the essential amino acids are not significantly racemized when processing involves exposure only to elevated temperatures. The combination of heat and alkali treatment, however, does cause racemization of those essential amino acids listed in Table 4.

Other examples of high D-aspartate contents in processed foods have been reported. Masters & Friedman (59) detected D-aspartate in several commercial food products, including texturized soy protein (9%), simulated bacon (13%), and nondairy creamer (17%). Finley (31) found significant D-aspartate in wheat crackers (9.5%), wheat cereal (11.0%), tortillas (11.6%), and hominy (15.4%).

Table 4 D-Amino acids in various foods (%)[a]

Processed food (Ref.) (Unprocessed control)	Asp	Ala	Phe	Leu	Val	Met
Toast[b] (14)	10.5	2.8	2.4	2.7	1.1	1.7
(Bread)	(5.6)	(2.4)	(2.3)	(3.2)	(0.9)	(2.3)
Extruded soy flour (14)	7.6	2.2	2.4	2.7	0.8	—
(Soy flour)	(4.4)	(2.5)	(2.8)	(1.4)	(1.0)	—
Soybean protein[c] (37)	27.7	9.9	19.7	3.1	1.0	18.2
(Untreated)	(0.5)	(0.2)	(0.1)	(0.2)	(0.03)	(0.3)
Taco shells (14)	5.8	3.2	—	3.8	1.5	1.1
(Corn meal)	(5.2)	(2.3)	—	(2.5)	(1.3)	(2.4)
Zein[d] (50)	40.2	17.6	31.3	5.0	2.9	19.5
(Unheated)	(3.4)	(0.7)	(2.2)	(0.7)	(0.4)	(0.9)
Hamburger[e] (14)	5.5	2.8	2.7	3.2	1.5	2.9
(Raw beef)	(6.2)	(3.2)	(2.8)	(3.1)	(1.6)	(2.4)
Chicken muscle[f] (55)	22.4	0.5	0.4	0.1	0	0
(Raw chicken)	(2.9)	(0)	(0)	(0)	(0)	—
Bacon 180°C[g] (40)	10.7	2.4	3.1	3.1	1.6	—
(Unheated)	(2.4)		(1.8)	(3.3)	(0.7)	—
Almonds 180°C[g] (40)	4.7	7.2	1.3	6.5	1.1	—
(Unheated)	(2.6)	(0)	(1.1)	(3.0)	(0)	—
Casein 230°C[g] (45, 46)	31.0	12.0	—	7.0	4.4	—
(Unheated)	(3.1)	(1.5)		(1.9)	(0)	
Soy nutrition liquid[h]	13.2	—		—	—	

[a] % D-amino acids = (D/D+L)100.
[b] White bread heated for 1 min, 45 sec. Analysis of surface only.
[c] 3 hours, 65°C, 0.1-N NaOH.
[d] 4 hours, 85°C, 0.2-N NaOH.
[e] Hamburger fried on both sides for 4 min. Pan surface 250°C. Only surface layer analyzed.
[f] Heated at 121°C, 4 hours.
[g] Roasted for 20 min.
[h] R. Chemburkar and E. H. Man (unpublished). Analysis and calculations same as in Table 3.

Included in Table 4 are data for fried hamburger, which indicate that insignificant racemization occurs during the cooking of this particular food. White bread toast, fried bacon, and chicken muscle that had been heated extensively contained appreciable levels of D-aspartic acid, which indicates that racemization takes place during the cooking of some foods.

Manufactured Proteins and Peptides

This category includes food substances ranging from completely synthetic products such as the sweetener aspartame (L-aspartyl-L-phenylalanine methyl ester) to products that are almost totally reconstituted proteins. Several of

these kinds of products are combined with carbohydrates as in some liquid diet mixes or simulated bacon. Although not actually dietary components, antibiotic peptides (9, 74) and some drugs used in chemotherapy (16) are greatly enriched in D-amino acid residues.

In all instances reported in the literature or examined by the authors, synthetic products were found to contain some of the highest levels of D-amino acids found in dietary sources. For example, a soy-protein-based liquid nutritive formulation purchased in a health food store was found to contain 13% D-aspartate (see Table 4), which is significantly higher than the values for soy-based infant formulas. Finley (31) reported that a commercially available weight loss product, which in error had been treated with excess alkali, contained 50% D-serine, 37% D-aspartate and 26% D-phenylalanine, and he suggested that this product could be dangerous if used as a sole source of dietary protein. Although such extreme values may be rare, this demonstrates the possibility of nearly racemic amino acids in food products exposed to alkali and/or heat for prolonged periods.

Boehm & Bada (10) studied racemization in the sweetener aspartame, and reported that both aspartic acid and phenylalanine are rapidly racemized at neutral pH and 100°C (see Table 1). Racemization occurs when the sweetener decomposes to its cyclic dipeptide (diketopiperazine), which is highly prone to racemization. These observations are important in view of the possible consumer misuse of the sweetener, such as in cooking.

METABOLISM

The examples given in the preceding section indicate that significant quantities of D-amino acids may be present in foods, and they demonstrate the universality of racemized amino acids in dietary substances. It has been known since the pioneering work of Krebs (53) that mammals possess enzymes specific for the metabolism of D-amino acids. D-amino acids are metabolized primarily via the D-amino acid oxidase pathway (7, 8, 15, 53, 54, 64). This reaction sequence is shown in Equation 3:

$$R\text{–}CH(NH_3^+)\text{–}COO^- + O_2 + H_2O \rightarrow R\text{–}CO\text{–}COO^- + NH_4^+ + H_2O_2. \qquad 3.$$

The α-keto acids can undergo stereospecific transamination yielding the L-enantiomer of the original amino acid, which is then metabolized by conventional pathways; or alternatively, they may be directly catabolized, e.g. via oxidative decarboxylation. The conversion of D-amino acids into α-keto acids takes place primarily in the kidney and liver, so ingested D-amino acids must first diffuse through membranes before they can be

metabolized via this pathway. Transport processes are themselves stereoselective and discriminate against D-amino acids (30, 41, 73).

The various D-amino acids are oxidized at different rates by D-amino acid oxidase. Some representative oxidation rates for the protein amino acids (relative to D-alanine) are given in Table 5. The D-enantiomer of aspartic acid, one of the amino acids most prone to racemization, is a poor substrate for D-amino acid oxidase. In mammals, however, there is a specific oxidase for D-aspartic acid but none for the other D-amino acids (26). Essential amino acids such as lysine and threonine that undergo racemization more rapidly than alanine (see Tables 1 and 2) are also very poor substrates for D-amino acid oxidase. Proline, which is not generally racemized to a significant extent during food processing (55), is one of the best substrates. There appears to be no correlation between susceptibility to racemization of an amino acid and its rate of oxidation by D-amino acid oxidase other than the existence of the enzyme specific for D-aspartate. The mammalian D-amino acid oxidase system thus does not appear to have developed in response to dietary racemized amino acids.

Krebs (53, 54) was unsure of the physiological function of D-amino acid oxidase. It is now generally believed that the role of D-amino acid oxidases in mammals is to detoxify "D-amino acids that are taken in as accidental contaminants, for example from the digestion of bacteria proteins" (6). This is supported by the observation that rats raised in a germ-free environment have a much lower activity of D-amino acid oxidases than those raised under normal conditions (6). However, D-glutamate, which occurs in bacterial cell wall proteins, is the poorest substrate for D-amino acid oxidase and is only

Table 5 Rates of oxidation, relative to alanine, of various D-amino acids by D-amino acid oxidase from sheep kidney [taken from the classic paper by Bender & Krebs (7)]

D-Amino acid	Relative oxidation rate	D-Amino acid	Relative oxidation rate
Alanine	1.0	Tryptophan[a]	0.53
Valine[a]	0.55	Lysine[a]	<0.01
Leucine[a]	0.22	Ornithine	0.05
Isoleucine[a]	0.34	Serine	0.66
Aspartic acid	0.02	Threonine[a]	0.03
Glutamic acid	not measurable	Methionine[a]	1.25
Phenylalanine[a]	0.41	Cysteine[a]	0.03
Tyrosine[a]	2.97	Proline	2.31
Histidine	0.10		

[a] An essential amino acid in humans (6).

slowly oxidized by D-aspartate oxidase (26). Although D-amino acid oxidases enable mammals to metabolize D-amino acids, the pathway is not efficient and can apparently be overloaded because, when racemic amino acids are ingested, a large fraction of the D-amino acids are excreted in the urine [see reviews by Neuberger (64) and Berg (8)].

Free D-amino acids may be converted by racemases into either racemic mixtures or their corresponding L-enantiomers. However, racemases primarily are found in bacteria and thus do not appear to be a major pathway in the metabolism of D-amino acids in mammals (6). There are D-amino acid transaminases, but these also appear to be restricted to bacteria (51).

The principal source of dietary D-amino acids for humans would be in the form of processed protein. Before these D-amino acids can be metabolized via the D-amino acid oxidase pathway they first must be liberated via proteolytic enzymes. Digestion of dietary proteins results in the formation of small peptides and free amino acids (6, 42). The peptides are hydrolyzed by peptidases (68, 72).

There is considerable evidence that peptides containing D-amino acid residues are resistant to hydrolysis by the enzymes involved with protein digestion. Studies with several synthetic small peptides indicate that D-aspartic acid (63) and D-methionine (65) are not liberated from peptides even though the other amino acid residues are L-enantiomers. Numerous reports have shown that heat- and alkali-treated proteins containing extensively racemized amino acids are resistant to proteolytic hydrolysis. Figure 1, for example, shows the decrease in protein digestibility versus the extent of racemization of the essential amino acid phenylalanine. This figure indicates that the phenylalanine racemization rate is twice the rate of loss in digestibility. Since phenylalanine has a slower racemization rate than aspartic acid, serine, and cysteine, it is apparent that even proteins that contain substantial racemized amino acids can be partly broken down by the proteolytic enzymes used in this assay.

The products of the proteolytic hydrolysis of proteins containing racemized amino acid residues are probably low-molecular-weight peptides enriched in D-amino acids. Di- and tripeptides can diffuse across membranes, whereas larger peptides apparently cannot and are probably simply excreted (61). The D-amino acids contained in di- and tripeptides are not good substrates for D-amino acid oxidase (15, 54). Dipeptides cyclize rapidly in vitro to diketopiperazines at neutral pH (76). Tripeptides rapidly hydrolyze nonenzymatically in vitro via an internal aminolysis pathway (77) to yield a cyclic dipeptide (diketopiperazine) and the free C-terminal amino acid. Amino acid residues in diketopiperazines may be highly susceptible to in vivo racemization (44, 76). Thus if this hydrolytic pathway occurred in vivo it could generate additional D-amino acids. The metabolic fate of D-amino acids in

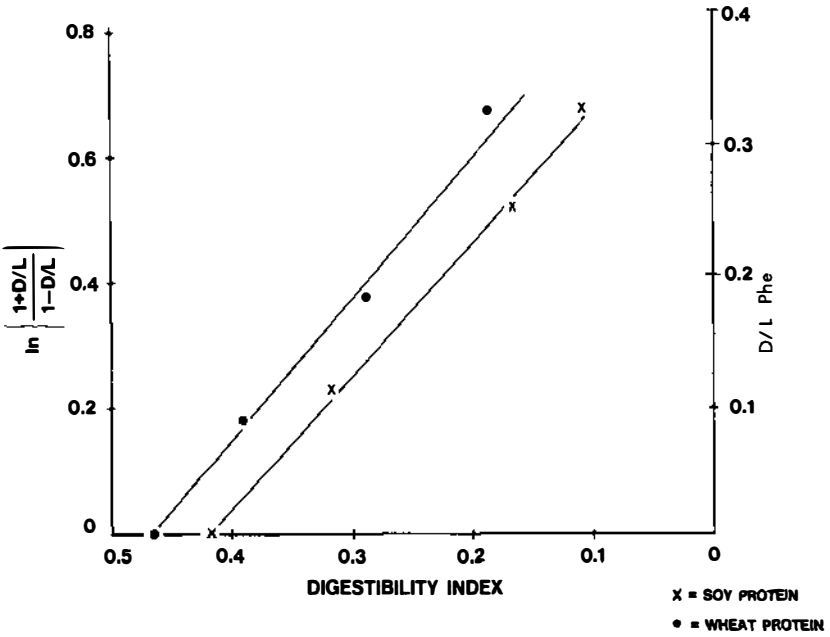

Figure 1 A plot of the extent of racemization of phenylalanine (in the form of Equation 2) vs the digestibility index of wheat and soybean proteins heated at 40°C in 0.2-N NaOH for various time periods. Taken from Chung et al (19). The digestibility index is defined as the fraction of the total peptide bonds hydrolyzed and was determined using an immobilized enzyme system with reactors containing pepsin, trypsin, chymotrypsin, and intestinal mucosal peptidases. The slope of the lines (≈ 2) gives the ratio of the phenylalanine racemization rate to the rate of loss of digestibility.

dipeptides and their cyclic diketopiperazine analogs is not known. Extensive studies of D-amino acids in small peptides are required in order to ascertain their metabolic fate.

NUTRITIONAL IMPLICATIONS

The long-term health consequences of racemized proteins in the human diet is not well known. As Masters & Friedman (59) pointed out, no studies have specifically addressed the nutritional consequence of racemization within human dietary proteins and how this affects digestibility and the metabolic accessibility of amino acids.

Deleterious Aspects

The utilization of protein-bound D-amino acids depends on whether D-amino acids can be liberated from L–D, D–L, or D–D peptide bonds in a food protein, and whether they can be efficiently transformed to their L-isomers (38). In the early part of this century Dakin & Dudley (23) first showed that when dogs

were fed alkali-treated casein, the protein was largely unabsorbed and was claimed to be recoverable in the feces. Extensive subsequent investigations have compared in vitro enzyme digestibility of alkali-treated vs untreated proteins. Reduced digestibility has been routinely observed for the treated samples, primarily caused by racemization and/or lysinoalanine formation.

Hayashi & Kameda (47, 48) studied the racemization of amino acid residues from alkali treatment of proteins, and observed the adverse effect on protein digestibility. They reported that even low levels of racemization caused an extensive decrease in protein digestibility. The loss of digestibility is explained by the fact that a racemized amino acid residue, which itself is not a substrate for proteases, can affect the reactivity of its nonracemized neighbors. Thus, the racemization of any particular amino acid residue could cause a significant loss of a neighboring essential amino acid as well as a decrease in the proteolytic digestibility of the protein itself.

Friedman et al (38) measured the in vitro digestibility of alkali-treated casein by trypsin and chymotrypsin as a function of temperature, time, and pH; they observed that digestibility of aspartate and phenylalanine decreased as both the extent of lysinoalanine cross-linking and racemization increased. The work of Bunjapamai et al (14) was the first successful attempt to separate the effects of racemization from cross-linking as measured by in vitro digestion. Their report concludes that the primary cause of reduced casein digestibility was racemization. Schwass et al (73) reported that if an amino acid residue is present in a peptide as the D-isomer, it can render that peptide unavailable for transport regardless of the amino acid involved. These authors conclude that racemization alone reduces in vitro digestibility as well as in vivo uptake of enzymatically digested protein, in concurrence with the findings of Bunjapamai et al (14).

An important question is whether dietary D-amino acids are toxic. From a general standpoint, the various L- and D-amino acids have similar acute toxicities as judged by their LD_{50} values (43). The exception may be D-proline, which was found to have a greater lethality than the L-enantiomer in chicks (18). As noted previously, D-proline is the best substrate for D-amino acid oxidase (see Table 5). Some D-amino acids apparently have long-term toxicities. This has been extensively discussed by Masters & Friedman (59), who noted that dietary D-serine, lysinoalanine, and various alkali-treated proteins have been reported to cause kidney lesions in rats. Free lysinoalanine was more nephrotoxic than peptide-bound lysinoalanine, and it has been suggested that in the alkali-treated dietary proteins used in the food industry, the lysinoalanine content is not nephrotoxically significant (31). Furthermore, rats seem to be especially prone to lysinoalanine-induced renal lesions (25), and there appears to be a species specificity for the nephrotoxic effects of dietary alkali-treated proteins and free lysinoalanine.

Lysinoalanine and alkali-treated proteins containing significant D-amino acid residues are in vitro inhibitors of several enzymes such as the carboxy- and amino-peptidases (39, 49). With lysinoalanine this inhibition results from the complexation of metal ion cofactors involved in the enzymatic reactions (49). Whether dietary D-amino acids and lysinoalanine are in vivo inhibitors of metabolic enzymes is not established, nor is the long-term effect of this inhibition.

Beneficial Effects

A decreased digestibility of dietary proteins enriched in D-amino acids may be advantageous from a dietetic standpoint, provided that components that remain after proteolytic digestion are not toxic. In fact, it may some day be possible to utilize racemized proteins in strategies for weight management. In addition, some D-amino acids have therapeutic properties. For example, D-phenylalanine and D-leucine have been shown to be analgesic (17) and have been used in the treatment of intractable pain (13). This analgesic effect is apparently due to the inhibition of enzymes such as carboxypeptidase A, which are involved in the degradation of the opioid pentapeptides present in the brain and spinal cord (13). Friedman et al (39) reported that alkali-treated food proteins that contained lysinoalanine and racemized amino acids also inhibited carboxypeptidase A. These results suggest that the presence of racemized amino acids in dietary proteins may be useful in pain inhibition.

It is now well established that most antibiotic peptides have D-amino acid sequences (9). It is thus conceivable that during the proteolytic breakdown of racemized dietary proteins, peptides may be generated that have antibiotic properties.

CONCLUSIONS AND FUTURE DIRECTIONS

The accumulated evidence on the metabolic effects of D-amino acids in nutritional proteins indicates that their presence reduces the digestibility as well as the availability of the component amino acids. The single most important source of D-amino acids in nutritional proteins is the processing that some foods undergo, either in cooking or as part of the manufacturing process used to prepare commercial food products.

Modern supermarkets contain ever increasing quantities of processed food products, including breakfast cereals, fried potato and corn chips, liquid and powdered infant formulas, and meat substitutes and extenders. The evidence that such products probably contain significant quantities of D-amino acids, coupled with the evidence that these D-amino acids most likely have deleterious or negative nutritional effects, suggests that further work in this area is needed relative to consumer safety, a point emphasized repeatedly by many

researchers. On the other hand, recent research indicates that certain D-amino acids may be beneficial. There may indeed be ways in which the limited digestibility of D-amino acids in dietary proteins can be utilized in nutrition, as for example in weight management or chronic pain control.

In this review we have considered only the D-enantiomers of the common protein amino acids. The D-enantiomers of some minor amino acids in foods should be examined since they may have important nutritional and therapeutic benefits. For example, dietary selenium partly in the form of L-selenomethionine may be a natural cancer inhibitor or anticarcinogen (62). Methionine and selenomethionine have similar racemization rates at 100°C in yeast (12). Based on the data we have presented here, this implies that selenomethionine would undergo significant racemization during the processing of some food proteins, which in turn might affect its biopotency.

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