REVIEWS

Chemistry, Nutrition, and Microbiology of D-Amino Acids

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Exposure of food proteins to certain processing conditions induces two major chemical changes: racemization of all L-amino acids to D-isomers and concurrent formation of cross-linked amino acids such as lysinoalanine. Racemization of L-amino acids residues to their D-isomers in food and other proteins is pH-, time-, and temperature-dependent. Although racemization rates of the 18 different L-amino acid residues in a protein vary, the relative rates in different proteins are similar. The diet contains both processing-induced and naturally formed D-amino acids. The latter include those found in microorganisms, plants, and marine invertebrates. Racemization impairs digestibility and nutritional quality. The nutritional utilization of different D-amino acids varies widely in animals and humans. In addition, some D-amino acids may be both beneficial and deleterious. Thus, although D-phenylalanine in an all-amino-acid diet is utilized as a nutritional source of L-phenylalanine, high concentrations of D-tyrosine in such diets inhibit the growth of mice. Both D-serine and lysinoalanine induce histological changes in the rat kidney. The wide variation in the utilization of D-amino acids is illustrated by the fact that whereas D-methionine is largely utilized as a nutritional source of the L-isomer, D-lysine is totally devoid of any nutritional value. Similarly, although L-cysteine has a sparing effect on L-methionine when fed to mice, D-cysteine does not. Because D-amino acids are consumed by animals and humans as part of their normal diets, a need exists to develop a better understanding of their roles in nutrition, food safety, microbiology, physiology, and medicine. To contribute to this effort, this multidiscipline-oriented overview surveys our present knowledge of the chemistry, nutrition, safety, microbiology, and pharmacology of D-amino acids. Also covered are the origin and distribution of D-amino acids in the food chain and in body fluids and tissues and recommendations for future research in each of these areas. Understanding of the integrated, beneficial effects of D-amino acids against cancer, schizophrenia, and infection, and overlapping aspects of the formation, occurrence, and biological functions of D-amino should lead to better foods and improved human health.

Keywords: *D-Amino acids; food processing; formation; microbiology; nutrition; safety*

INTRODUCTION

With some exceptions, each amino acid of importance in nutrition has one asymmetric C atom that can exist in two mirror images (D and L configurations), called enantiomers. The two amino acids with two asymmetric C atoms (Ile and Thr) can exist as four diastereoisomers. Generally, only L-amino acids are susceptible to enzyme-catalyzed polymerization (translation) to structural and functional peptides and proteins. L-Amino acid residues in food proteins are subject to racemization to D-isomers under the influence of food processing conditions, especially heat and alkaline pH (Friedman et al., 1984a,b). Although proteins containing D-amino acids can be hydrolyzed at peptide bonds containing L-amino acids, the hydrolysis rates may be slower than those for corresponding native proteins. Such changes can impair the nutritional quality and safety of foods by generating nonmetabolizable and biologically nonutilizable forms of amino acids, creating D−D, D−L, and L−D peptide bonds partly or fully inaccessible to proteolytic enzymes and forming nutritionally antagonistic as well as toxic compounds. Furthermore, these altered proteins may compete with proteins that do not possess racemized amino acids for the active site of digestive proteinases in the gut and thus render the unracemized proteins also less nutritionally available. It is not known whether D-amino acid-containing oligopeptides can change the microbial flora of the digestive tract.

Since the early part of this century (Dakin and Dudley, 1913; Kossel and Weiss, 1909; Levene and Bass, 1927), alkali treatments have been known to racemize amino acids. The field of amino acid racemization in proteins lay largely dormant for about half a century because of lack of suitable analytical methods to measure the formation of specific protein-bound D-amino acids. The development of gas chromatographic (GC) and high-performance chromatographic (HPLC) techniques along with chiral columns that can separate D- and L-isomers stimulated widespread interest in this subject.

As a result of food processing using these treatments, D-amino acids are continuously consumed by animals

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Table 1. Foods Containing D-Amino Acids (Listed Alphabetically)

alcoholic beverages
beer, wines (white, red), sake, vinegars
bacterial starter cultures
baked products
bread (fresh, toasted), crackers, dough, wheat flour
bean products (legumes)
black beans (fermented), soy flour (extruded), soy infant formula, soy protein (texturized), soy sauces and pastes, soybeans (fermented, miso)
cacao powder
coffee
cream (sodium caseinate), green, instant, roasted
corn meal tacos and tortillas
dairy products
cheeses
aged (ripened), Cheddar, fresh, baked, Italian (mozzarella, Parmesan), Swiss (Emmentaler)
milks and fermented milks
bacon (simulated), buttermilk, cream (imitation), dips (bacon and Cheddar), milk (cows, mastitis cows), curdled, fermented (kefir, yakult, yogurt), goats, human, infant formulas, mares, sows, pasteurized, powder, raw, sheep, sour cream, whey (dietary)
eggs
fish meal
food colorants
fruits and vegetables
apples, carrots, cabbage (white, green, pickled, red), garlic, grapefruits, grapes, lemons, oranges, tomatoes
fruit and vegetable juices
apricot, beet, blackberry, cabbage (pickled), carrot, celery, cherry, cranberry, currant, nectarine, orange, peach, pear, raspberry, sauerkraut, tomato (paste, puree, ketchup), strawberry
honey
meat and meat products
bacon (unheated), beef and chicken soups, chicken (raw), ham, hamburgers (baked, fresh), sausage (fermented)
sauces
mustard, pepper, soy
soups
spices and flavor enhancers
vinegar
yeast extract

[a] See text and the following references for reported D-amino acid values: Albertini et al., 1996; Brückner and Hausch, 1989, 1990a,b; Brückner and Lüpke, 1991; Brückner and Westhauser, 1994; Brückner et al., 1993, 1995; Calabrese et al., 1995; Chang et al., 1999a,b; Chiavaro et al., 1998; Csapo et al., 1995; Kato et al., 1995; Luzzana et al., 1996; Marchelli et al., 1992; Master and Friedman, 1980; Friedman, 1999; Friedman et al., 1981; Gandolfi et al., 1992; Gobbetti et al., 1993, 1994; Jin et al., 1999; Kuneman et al., 1988; Lee et al., 1995; Mann and Bada, 1987; Palla et al., 1989; Pawlowska and Armstrong, 1994; Rhee et al., 1994; Rundlett and Armstrong, 1994; Sato et al., 1997; Schormüller and Weder, 1962.

and humans. Heat alone can also racemize amino acids in proteins. For example, Hayase et al. (1973, 1975, 1979) observed racemization of eight amino acids in roasted casein and bovine serum albumin. In addition, D-amino acids are synthesized in vivo by microorganisms and other living organisms by transformations of L-isomers catalyzed by the enzymes amino acid oxidases, transaminases, and epimerases (racemases). Because D-amino acids are formed during food processing and also originate from microbial sources and aqueous, soil, and other environments and may become part of our diet (Table 1), a need exists to assess the factors that influence their formation in food and other proteins, their biological function and utilization by animals and humans, their safety, and their role in antimicrobial peptides, bacterial cell wall components (peptidoglycans), and other functional and structural biomolecules.

This integrated overview describes factors that influence racemization of L-amino acid residues in food proteins, the distribution of D-amino acids in the food chain and various organisms, D-amino acids as indicators of food quality, the biological utilization of free and protein-bound D-amino acids, their impact on nutrition and food safety, and possible roles of D-amino acids in microbiology and other biomedical sciences. This paper is intended to complement earlier assessment of the dietary significance of lysinoalanine and other cross-linked amino acids, the formation of which takes place concurrently with racemization, and of the dietary roles of tryptophan, sulfur amino acid, food proteins, and enzymatic and nonenzymatic browning reactions published in this journal (Friedman, 1994, 1996a,b, 1997, 1999; Friedman and Cuq, 1988). A better comprehension of these overlapping major chemical events is needed to define some of the parameters that impact food quality and safety, nutrition, and microbiology. Such an understanding will make it possible to minimize adverse effects of food processing and maximize beneficial ones.

CHEMISTRY OF RACEMIZATION

Origin of Chirality. Although the formation of L-amino acid and D-sugars in the evolution of life is generally considered to be a result of chance (Bonner, 1998; Isoyama et al., 1984; Miller and Orgel, 1974; Prelog, 1976), Mason and Tranter (1984) describe an intriguing mathematical model, the parity-violating energy differences between enantiomeric molecules, which predicts that an enantiomeric excess is expected in chiral products resulting from reactions of achiral or racemic substrates. Thus, a nonequilibrium racemic reaction system such as the polymerization of D,L-amino acids to a protein spontaneously switches to a homochiral reaction channel, giving only the L-product. Calculations show that polypeptides of the L-series are preferentially stabilized by electroweak interactions. Such polypeptides have lower energies due to parity violation than the corresponding D-isomers. Because the formation of optically active biomolecules may be "energy-driven", the creation of homochiral biochemistry may not be accidental.

Analysis of D-Amino Acids. Because all of the amino acid residues in a protein undergo racemization simultaneously, but at differing rates, assessment of the extent of racemization in a protein requires quantitative measurement of ~40 L- and D-optical isomers. Analytically, this is a difficult problem. Numerous studies have attempted to optimize separation of derivatized amino acids on various chiral columns followed by analysis by GC, GC/MS, and liquid HPLC. The following references offer a useful entry into the literature on this subject: Armstrong et al., 1991, 1993a; Brückner et al., 1994a,b; Cavins and Friedman, 1968; Ekborg-Ott and Armstrong, 1997; Engel et al., 1981; Frank et al., 1978; Jin et al., 1999; Küsters et al., 1984; Liardon and Lederman, 1983; Liardon et al., 1991; Liu et al., 1997; Marchelli et al., 1996; Sacchi et al., 1998; Sarwar et al., 1983; Varadi et al., 1999; Zhujun et al., 1995.

Stereochemistry of Amino Acids. Figure 1 shows the structures, names, and abbreviations of most of the amino acids discussed in this paper. The following description of the stereochemistry of the protein amino acids is adapted from Eggum and Sorensen (1989) and Friedman (1977). The absolute configuration at carbon atom 2 (C-2) of the optically active amino acids is designated with the prefixes D- and L-, which correlate

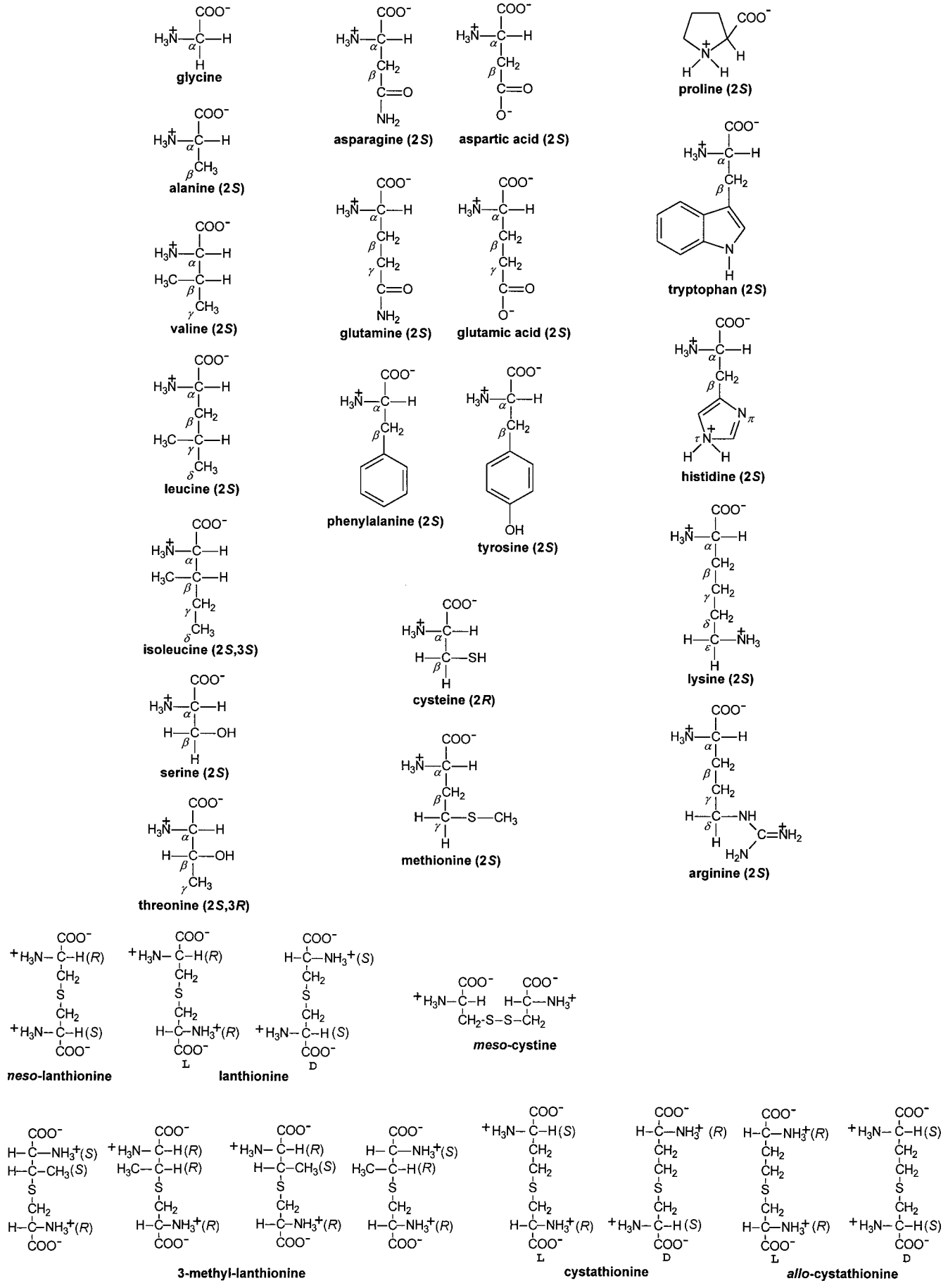

Figure 1. Structures, configurations, and names of genetically coded protein amino acids and post-translationally formed sulfur amino acids. For analogous designations of lysinoalanine and methyl-lysinoalanine isomers, see Friedman (1977, 1999).

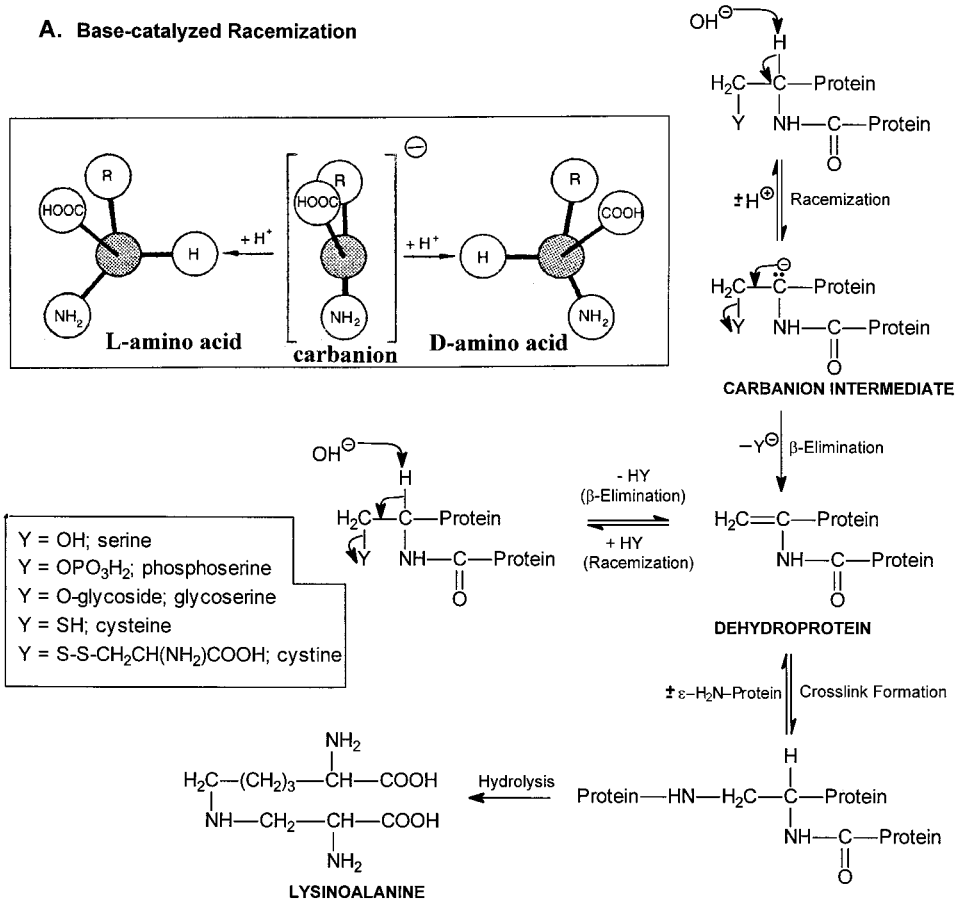

Figure 2. (A) Base-catalyzed racemization: (a) Proton abstraction−addition mechanism. An OH^- ion abstracts an H^+ from the α-CH of an amino acid to form a negatively charged carbanion, which has lost its original asymmetry [inset, adapted from Zagon et al. (1991a,b)]. The carbanion can then recombine with a proton from the solvent to regenerate the original amino acid which is now racemic (DL). (b) Elimination−addition mechanism. The carbanion can also undergo an elimination reaction to form dehydroalanine side chain. The dehydroprotein can then react with active-hydrogen bearing functional groups (e.g., HY = H_2O) to form a racemized amino acid side chains (e.g., DL-Ser) (Friedman et al., 1981; Sawyer et al., 1983). The concurrent formation of lysinoalanine is also shown. (B) Acid-catalyzed racemization. Protonation of the carboxyl group of an L-amino acid facilitates removal of the α-CH proton to form the asymmetric dehydroalanine. Proton addition at two sides (*re* and *si* faces) of the double bond generates both D- and L-isomers (Frank et al., 1981; Liardon et al., 1983, 1991).

with D- or L-Ser and L- and D-glyceraldehyde, respectively. All protein amino acids except glycine, which has no asymmetric C atom, are of the L-form. They thus have the (2*S*)-configuration according to the *R*,*S*-system, provided a sulfur atom is not present at C-3, as is the case with cysteine. Because the sulfur atom changes the priorities of the substituents at the chiral center, L-cysteine has the (2*R*)-configuration.

L-Thr and L-Ile have two asymmetric C atoms, and each can exist in four diastereoisomeric configurations: L-Thr, D-Thr, L-*allo*-Thr, D-*allo*-Thr; L-Ile, D-Ile, L-*allo*-Ile, D-*allo*-Ile. Four diastereoisomers are possible for 4-hydroxyproline, two *cis* and two *trans*.

Racemization Mechanisms. Parts A and B of Figure 2 depict base and acid catalysis of racemization, and Figure 3 depicts isomeric inversions in vivo. In our seminal study on the base-catalyzed racemization of amino acids in alkali-treated casein, lactalbumin, soy protein, and wheat gluten, we (Masters and Friedman, 1979) delineated the factors that govern racemization of protein-bound amino acid residues. We found that the rate of racemization is first-order with respect to OH^- concentration. Although racemization rates of individual amino acids vary among the four proteins, the relative rates in any given protein are similar. We also appear to have been one of the first to report the presence of D-amino in commercial foods (Masters and Friedman, 1980; Friedman et al., 1981). These findings stimulated extensive studies on the dietary and biological significance of racemization of food and other proteins.

Racemization of an amino acid proceeds by removal of a proton from the asymmetric C−H bond of an amino

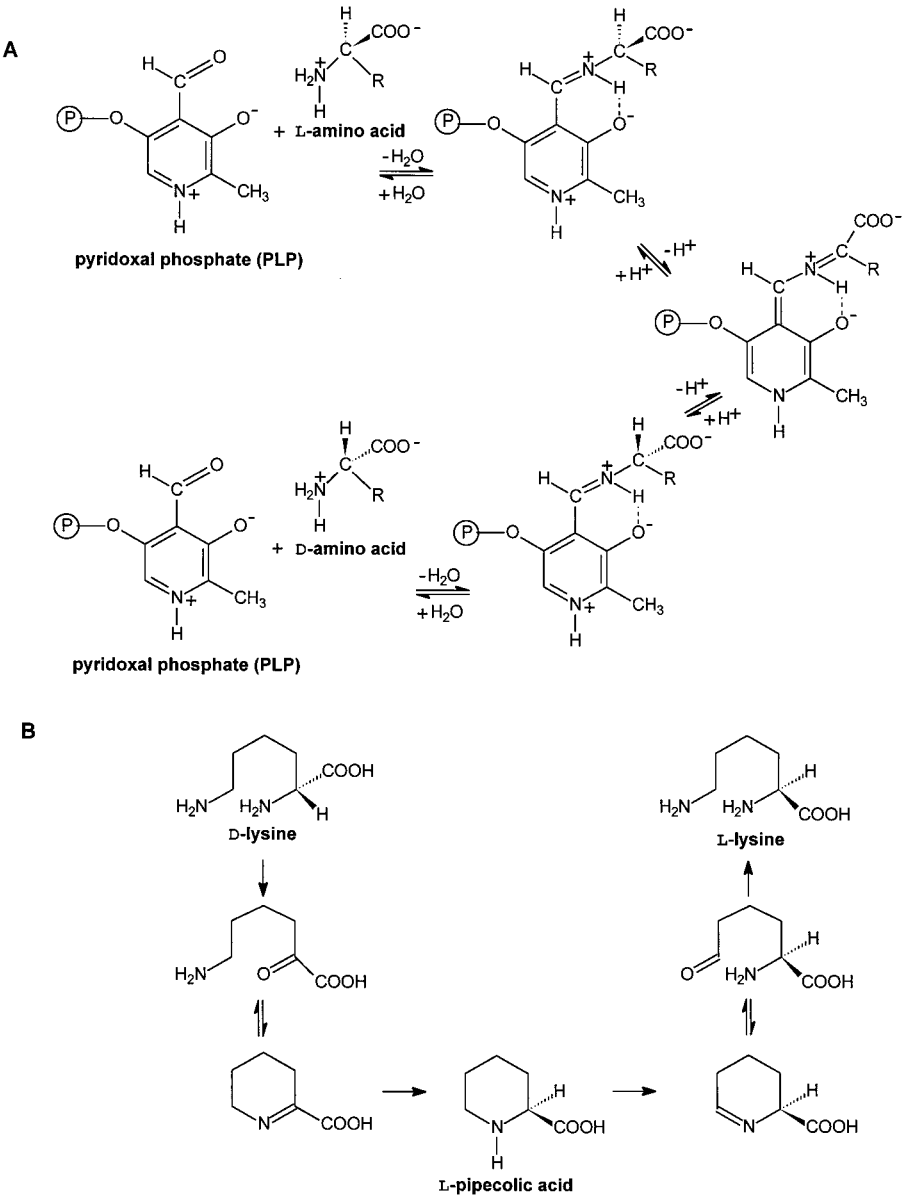

Figure 3. Isomeric inversion in vivo: (A) catalysis of inversion of an L-amino acid by the pyridoxal phosphate (PLP) coenzyme of a microbial racemase involving stereoselective shifts of H atoms [adapted from Soda (1996)]; (B) conversion of D- to L-lysine by plant and microbial enzymes via pipecolic acid [adapted from Fangmeier and Leistner (1980, 1981)].

acid residue to form a carbanion intermediate (Figure 2A). The trigonal carbon atom of the carbanion, having lost the original asymmetry, then recombines with a proton from the solvent to regenerate a tetrahedral structure. The reaction is written as

$$\text{L-amino acid} \underset{k'}{\overset{k}{\rightleftharpoons}} \text{D-amino acid} \qquad (1)$$

where k and k' are the first-order rate constants for the forward and reverse racemization rates, respectively.

The product is racemic if recombination can take place equally well on either side of the carbanion, giving an equimolar mixture of the L- and D-isomers. However, recombination may be biased if the molecule has more than one asymmetric carbon center, resulting in an equilibrium mixture somewhat different from the 1:1 enantiomeric ratio. For amino acid residues with one asymmetric C atom, the maximum amount of racemization produces an equimolar mixture of L- and D-isomers regardless of whether one begins with an L- or a D-amino acid residue. The extent of racemization is usually reported as %D = [(D/D + L) × 100] and/or absolute concentration of the D-amino acid in the sample, usually milligrams per gram.

The following operational equation, whose derivation is given in Masters and Friedman (1980), is useful to relate the calculated apparent racemization rate constant (k) to the extent of measured racemization (D/L ratio) for each amino acid during a specific time period, for example, 3 h:

$$k = \ln[(1 + \text{D/L})/(1 - \text{D/L})]_{(\text{reaction time})} - \ln[(1 + \text{D/L})/(1 - \text{D/L})]_{(\text{initial time})} \qquad (2)$$

Factors that favor racemization include increased pH of the medium, reaction temperature, and time. Table 2 and Figures 4 and 5 show racemization trends for selected proteins as influenced by these parameters. In addition, the extent of racemization of a number of amino acid residues in eight food proteins as well as

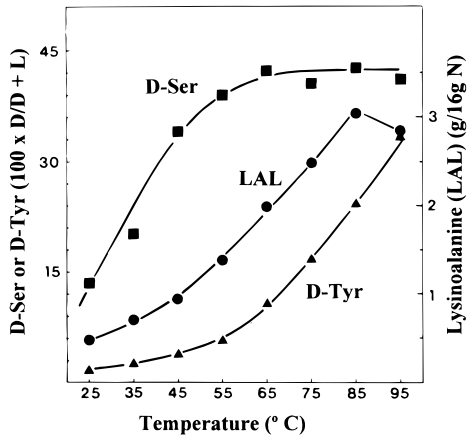

Figure 4. Effect of temperature on D-Ser, D-Tyr, and lysinoalanine content of soybean protein [prepared from data given in Friedman and Liardon (1985)].

several polyamino acids evaluated also appears to have been influenced by concurrent peptide bond cleavage, as measured by a ninhydrin assay (Liardon and Friedman, 1987). Will stereochemically different D-amino acids and D-amino acid-containing peptides and proteins react with ninhydrin at rates different from those of the corresponding L-analogues (Friedman and Sigel, 1966; Friedman and Williams, 1974a,b; Friedman et al., 1979, 1984c; Pearce et al., 1988)?

Table 2. Enantiomeric Composition (Percent D) of Eight Alkali-Treated Proteins[a]

amino acid	casein	lactal-bumin	wheat gluten	zein	fish	soy-bean	bovine albumin	hemo-globin
Ala	15.2	14.4	18.6	22.2	19.3	15.8	22.1	17.1
Val	2.6	2.7	4.0	4.9	3.1	2.5	3.5	4.0
Leu	7.4	5.0	7.2	7.8	6.8	6.3	8.2	6.6
Ile	3.3	3.1	4.0	5.5	3.6	3.9	5.7	5.0
Cys		32.1	32.0	43.7	22.8	21.0	23.0	30.0
Met	24.7	32.3	33.1	29.8	29.2	24.3	30.0	26.2
Phe	24.4	24.3	24.4	32.4	28.0	25.5	28.1	30.0
Lys	8.1	7.2	9.4	8.0	11.5	11.3	13.3	9.9
Asp	29.2	22.6	25.6	41.6	25.0	30.8	27.0	18.9
Glu	19.7	19.5	32.3	35.0	18.9	21.1	18.4	19.8
Ser	41.0	47.1	42.2	44.0	42.1	44.2	43.0	44.5
Thr	29.3	29.1	30.0	36.3	32.8	27.8	28.3	31.2
Tyr	15.0	18.9	19.5	35.5	16.3	13.7	15.3	22.6
LAL[b]	4.4	5.4	0.9	0.3	2.8	3.2	8.5	4.4

[a] Conditions: 0. 1 N NaOH; 75 °C; 3 h (Friedman and Liardon, 1987; Liardon and Friedman, 1987; Liardon et al., 1991). [b] Mixture of (LD + LL) lysinoalanine isomers in grams per 16 g of N.

Because structural and electronic factors that facilitate the formation and stabilization of the carbanion intermediate are unique for each amino acid, it follows that the reaction rate for the isomerization of each amino acid is also unique and differs from protein to protein. Thus, the inductive strengths of each amino acid side chain [R substituent in $R-CH(NH_2)COOH$]

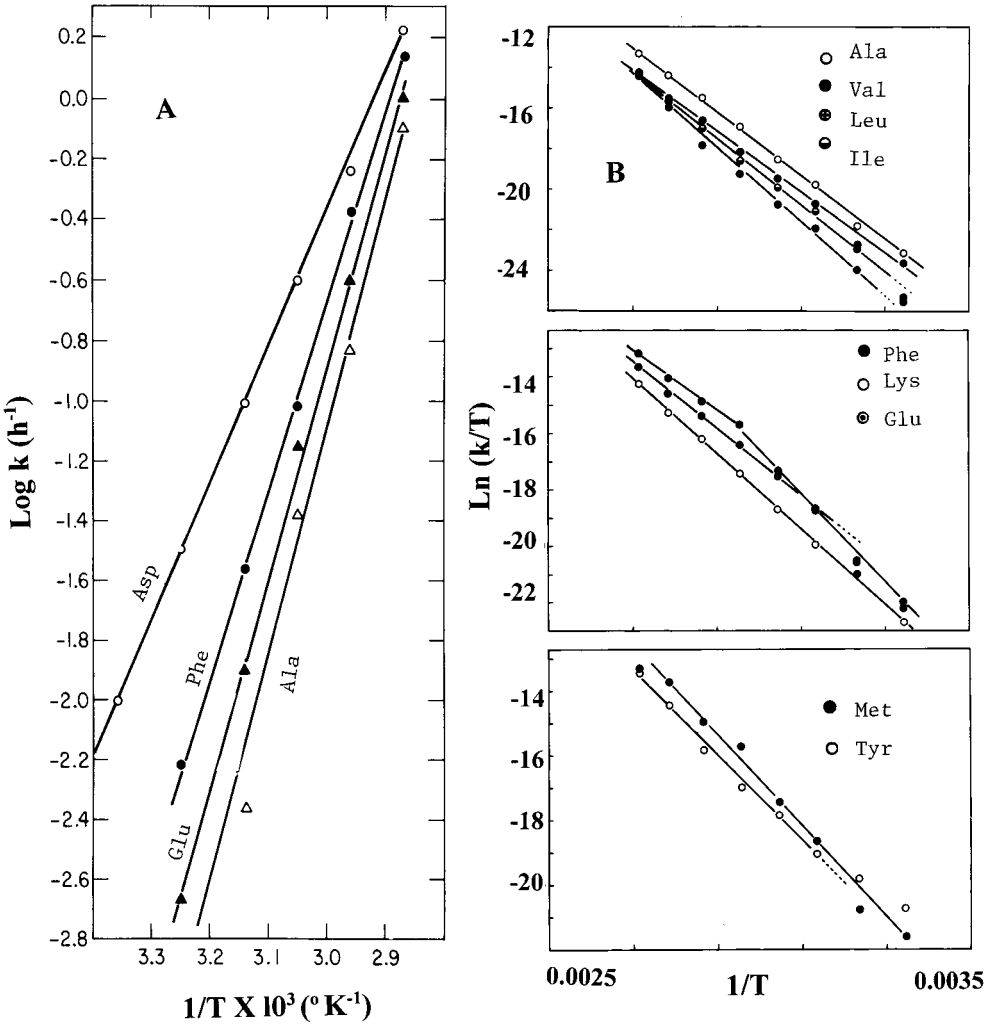

Figure 5. Arrhenius plots for the racemization of protein amino acid residues in proteins: (A) casein (Friedman and Masters, 1982); (B) soy protein (Friedman and Liardon, 1985). Reprinted with permission. Published 1982 by the Institute of Food Technologists (A) and 1985 by the American Chemical Society (B).

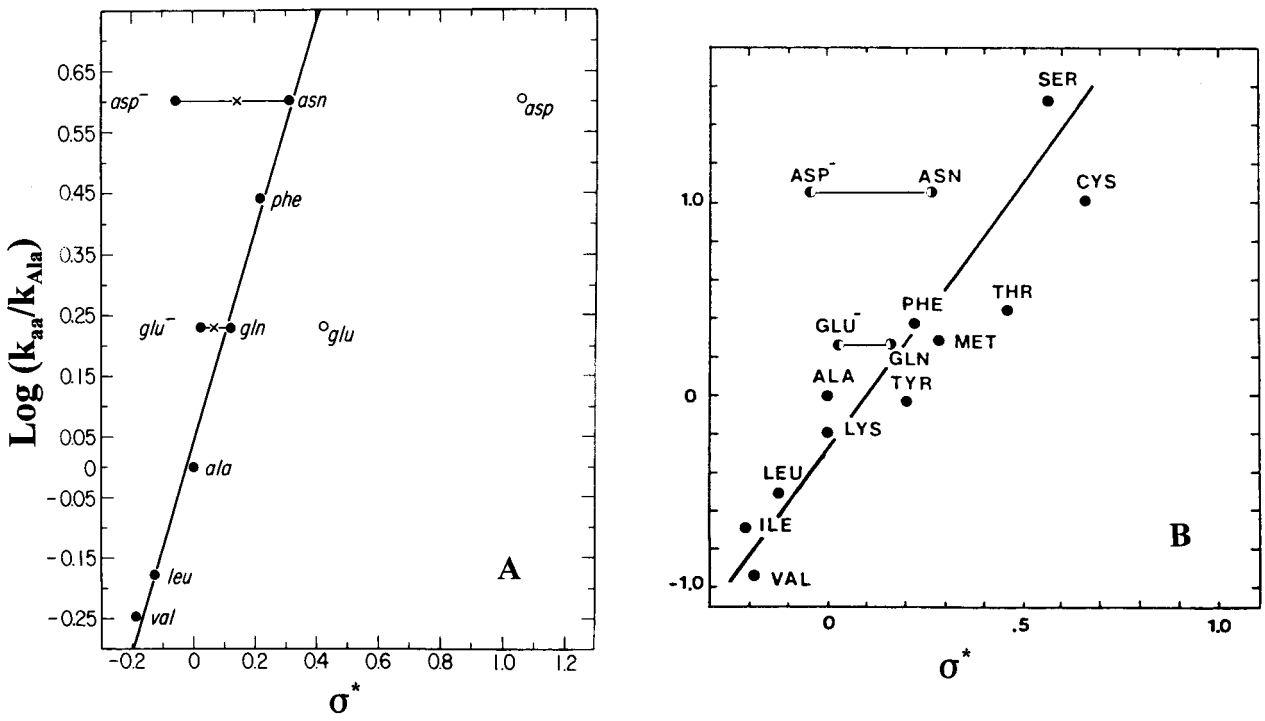

Figure 6. Relationship between the inductive constant (σ^*) of the amino acid side chain R in $RCH(NH_2)COOH$ and the racemization rate constant (k_{aa}) for the indicated amino acids relative to Ala (k_{ala}): (A) casein (Friedman and Masters, 1982); (B) soy protein (Friedman and Liardon, 1985). Reprinted with permission. Published 1982 by the Institute of Food Technologists (A) and 1985 by the American Chemical Society (B).

have been invoked to explain differing racemization rates of amino acids in food proteins as influenced by pH, time, and temperature. Plotting racemization for individual amino acids in casein and soybean proteins demonstrates good correlation between the inductive constants for the R groups and racemization rates (Figure 6). These linear free energy relationships support the described mechanism of racemization. Moreover, because *relative* racemization rates of all amino acid residues within any protein appear to remain invariant, these relationships may also be useful for predicting racemization rates for all amino acid residues of a protein from an experimentally determined rate for one amino acid, for example, alanine.

Acid-catalyzed racemization, which occurs at a much slower rate than the base-catalyzed process, involves protonation of the carboxyl group of the amino acid followed by the indicated equilibria to form the asymmetric dehydroalanine. The latter then regenerates an equimolar mixture of D- and L-isomers (Figure 2B; Frank et al., 1981; Liardon et al., 1991).

The mechanism for radiation- and hydroxyl ($OH^{\bullet}$)- or other radical-catalyzed racemization probably involves homolytic cleavage of the α-C−H bond of an amino acid to generate the asymmetric radical [$R-C^{\bullet}(NH_2)COOH$]. The latter can then extract a hydrogen atom ($H^{\bullet}$) from the environment to produce a mixture of isomers. This aspect merits further study in view of the current interest in the use of radiation to kill microorganisms in foods (Bonner and Blair, 1979; Swallow, 1991).

Heat-catalyzed racemization of protein-bound L- to D-Asp takes place via formation of an aspartyl succinimide intermediate, tautomerization, and release of D-Asp after protein hydrolysis (Geiger and Clarke, 1987; Lüpke and Brückner, 1998). Elucidation of the mechanism of heat-catalyzed racemization of other protein-bound amino acids (Fuse et al., 1984) awaits further study.

Heating optically active α-amino acids in the presence of aromatic aldehydes (Ar-CHO) such as benzaldehyde induces racemization via stereospecific formation of 1,3-dipolar intermediates of the following structure: [Ar—CH═NH^+—C^- (R)COOH] (Grigg and Gunaratne, 1983). An analogous mechanism has been proposed for the microbial racemase-catalyzed inversion of L- to D-amino acids (Soda, 1996). In this case, it is the aldehyde group of the pyridoxal moiety of the racemase that forms the dipolar intermediate (Figure 3A). This mechanism is distinct from that operating in the amino acid oxidase-catalyzed transformations of D- to L-amino acids involving transaminations (Sugio et al., 1993; Yoshimura et al., 1993).

In a related study, Friedman et al. (1974) showed that because it can be used at neutral pH, sodium cyanoborohydride may be a better reagent to reduce the double bond of aldehyde derivatives of proteins to the corresponding amines than sodium borohydride (which is active at alkaline pH). This reductive alkylation reaction may facilitate mechanistic studies on aldehyde-catalyzed racemization. These considerations also suggest that aldehyde-containing food ingredients such as glucose and glyceraldehyde could in principle catalyze racemization of amino acids by a similar mechanism.

Figure 3B shows a proposed mechanism of enzyme-catalyzed inversion of D- to L-lysine via cyclization to a pipecolic acid intermediate that occurs in plants, bacteria, fungi, and mammals (Fangmeier and Leistner, 1980, 1981). The biological significance of pipecolic acid isomers is mentioned below under Role of D-Amino Acids in Disease.

Discriminating between Racemization and Lysinoalanine Formation. Because alkali-induced racemization of protein amino acids is accompanied by lysinoalanine formation, nutritional and related changes resulting from consumption of such proteins arise as a consequence of both chemical events. Because acetyla-

tion of ϵ-NH_2 groups of lysine side chains of casein and soy proteins was found not to affect the nutritional quality of these proteins but did prevent lysinoalanine formation when the acetylated proteins were exposed to high pH, it is possible to prepare racemized proteins without lysinoalanine (Friedman, 1978; Friedman et al., 1984a,b; Master and Friedman, 1980). Feeding studies with alkali-treated native and acetylated proteins should make it possible to discriminate between biological consequences of racemization from lysinoalanine formation.

DISTRIBUTION OF D-AMINO ACIDS IN FOOD

Reporting the presence of D-amino acids in processed foods, Masters and Friedman (1980) found that the percent of D-Asp of the total Asp was 9 for textured soy protein, 10 for an infant soy formula, 13 for simulated breakfast strip bacon, and 17 for an imitation coffee cream whitener. This observation was followed by numerous studies, summarized in Table 1, on the content of D-amino acids in a variety of foods. The possible origin of these D-amino acids and their dietary significance will be examined in this section.

Dairy Products. Raw milk from ruminants (cows, goats, and sheep), but not human milk, contains the following D-amino acids: D-Ala, D-Asp, D-Glu, D-Lys, and D-Ser (Gandolfi et al., 1992; Lee et al., 1995; Schormüller and Weder, 1963). In contrast, milk from mares contains only D-Glu and D-Lys (Albertini et al., 1996). These differences could be due to the fact that D-amino acids in cow's milk originate from the digestion (autolysis) of peptides and proteins containing D-amino acid originating from microbial cell walls (peptidoglycan) proteins in the rumen of the cows (Rooke et al., 1984; Schleifer and Kandler, 1972), whereas the origin of the D-amino acids in the other milks may arise from a different, unknown physiological event.

The use of various microorganisms to ferment milk into a variety of dairy products significantly enhances their D-amino acid content and results in the appearance of additional D-amino acids (Palla et al., 1989). For example, Brückner and Hausch (1989, 1990a,b) found that in addition to D-Ala (percent D-isomer relative to L-isomer = 61.3), D-Asp and D-Glu were present in yogurt. Kefir and curdled milk also contained relatively high concentrations of D-Val, D-Leu, D-Ile, and D-Ser. Relatively high concentrations of these D-amino acids were are also present in widely consumed ripened cheeses such Gouda, Emmentaler, and Parmesan. The differences may arise from the use of different microorganisms in the fermentation process (Brückner et al., 1992, 1993).

The fact that the D-Ala content of raw milk increased during storage at 4 °C implies that D-Ala could serve as an indicator of contamination of milk by D-Ala-producing psychotrophic bacteria. Because mastitis is an infection of the udder caused by bacteria, milk from infected cows should have a higher D-amino acid content originating from the microorganisms. This is indeed the case (Csapo et al., 1994, 1995). Pasteurization of milk does not seem to increase its D-amino content.

Partial hydrolysis of casein in milk and cheese often leads to the accumulation of bitter peptides. In an attempt to overcome this problem, Gobbetti et al. (1994) explored the use of proteases present in *Pseudomonas fluorescens* to hydrolyze such peptides. The cell-associated peptidase activity resulted in a higher D-amino content in the treated compared to the untreated milk. The additional D-amino acids presumably originate from this microorganism.

The report by Lubec (1989) that microwave heating of milk infant formulas induces formation of D-proline and *cis*-L-hydroxyproline compared to conventional heating could not be confirmed (Fay et al., 1991; Fritz et al., 1992; Marchelli et al., 1992; Petrucelli and Fischer, 1994). It is also noteworthy that microwave heating of gelatin did not generate *cis*-4-hydroxy-L-proline, an inhibitor of collagen biosynthesis (Erbe and Brückner, 1999).

Sourdough Fermentation. Use of lactic acid bacteria and yeast in the fermentation of sourdough before baking results in the introduction of D-Ala and D-Glu into the dough (Gobetti et al., 1994). Baking of the dough into bread induces a 44% decrease in the total free D-amino acid content. No abiotic formation of D-amino acid was noted as a result of exposure of the dough to heat during baking. In contrast to bread, roasted coffee contained 10−40% of D-Asp, D-Glu, and D-Phe (Palla et al., 1989).

Fruits and Vegetables. Fruits (apples, grapes, oranges) and vegetables (cabbage, carrots, garlic, tomatoes) as well as the corresponding juices contain variable but measurable amounts of D-amino acids including D-Ala, D-Arg, D-Asp, and D-Glu (Brückner and Westhauser, 1994; Gandolfi et al., 1994). These amino acids could originate from plant sources, from soil and other microorganisms, and/or from heat treatments used to pasteurize juices. Whether the presence of specific D-amino acids could permit differentiating juices from biologically dissimilar fruits and whether D-amino acids could serve as an indicator for detecting bacterial activity and shelf life of fruit juices merit further study (Gandolfi et al., 1994).

D-Glutamic Acid in Processed Foods. The sodium salt of the naturally occurring nonessential amino acid monosodium glutamate (MSG) is often added at levels of 0.2−0.9% to foods to improve flavor and palatability. An extensive survey by Rundlett and Armstrong (1994) showed that a variety of processed foods contain significant amounts of D-Glu (which does not possess flavor-enhancing properties). The %D ranged from 0.25 for pure MSG to 0.8 for soups (highest value), 2.9 for crackers, 7.9 for sauces, 18 for vinegars, 36 for sauerkraut juices, 1.6 for tomato products, and 6.2 for milk products.

The D-Glu could originate from microbial sources as well as from food-processing-induced racemization of free and protein-bound L-Glu. Table 2 shows that protein-bound L-Glu is one of the fastest racemizing amino acid. These considerations suggest that a need exists to ascertain both free and protein-bound D-Glu to make reasonable conclusions about the dietary significance of D-Glu in processed foods, in view of the fact that certain individuals are reported to suffer from migraine-like headaches ("Chinese Restaurant Syndrome") following consumption of MSG. The possible impact of the D-isomer of MSG on this problem has apparently not been evaluated. It should be noted, however, that the question of whether MSG or other food ingredients [such the biogenic amines histamine and phenylethylamine (Friedman and Noma, 1981, 1986)] are responsible for the reported symptoms has apparently not yet been resolved (Anderson and Raiten,

1992; Anonymous, 1995; Tarasoff and Kelly, 1993; Samuels and Samuels, 1993).

Eggs. Alkali pickling of duck eggs in a 4.2% NaOH/5% NaCl solution for 20 days at room temperature used to prepare the traditional Chinese pidan resulted in extensive racemization of amino acid residues and concurrent formation of lysinoalanine (Chang et al., 1999a,b). The relative racemization rates appear to be similar to those observed with egg albumin and other pure proteins listed in Table 2. Possible nutritional consequences of the observed changes in amino acid composition merit study.

Honey. The D/L ratios of Leu, Phe, and Pro could serve as indicators of age, processing, and storage histories of honeys (Pawlowska and Armstrong, 1994).

Fish Meal. Luzzana et al. (1996) found that heating of laboratory-made herring meals at 125 °C induced time-dependent formation of D-Asp. The D-Asp acid content of 12 fish meals from various sources ranged from 0.7 to 3.7% D. The D-Asp content may indicate the severity of the thermal treatment during cooking or drying of fish meals.

Other Foods. Variable amounts of D-amino acids have been reported to be present in other fermented and processed foods including beer, vinegar, wine, bread toast, hamburger meat, ham, liquid spices, powdered milk, soy paste, and soy sauce (Brückner et al., 1995; Chiavaro et al., 1998; Ekborg-Ott and Armstrong, 1996; Friedman et al., 1981; Man and Bada, 1987; Rhee et al., 1994; Zagon and Dehne, 1994; Varadi et al., 1999; Zagon et al., 1991a,b).

NATURAL OCCURRENCE AND FUNCTION OF D-AMINO ACIDS IN PLANTS, MICROBES, AND HIGHER ORGANISMS

Plants. Plants appear to be able to synthesize D-amino acid derivatives and peptides de novo (Fangmeier and Leistner, 1981; Ogawa, 1978; Robinson, 1976), as illustrated by the following examples. Germinating pea seedlings (*Pisum sativum*) contain high concentrations of *N*-malonyl-D-Ala and γ-L-glutamyl-D-Ala (Fukuda et al., 1973). The concentration of the latter increased to 2.5 μM/seedling after 8 days of germination. Noma et al. (1973) isolated D-Ala-D-Ala from tobacco leaves. The relative concentrations of three D-Ala peptides (D-Ala-Gly, D-Ala-D-Ala, and D-Ala-L-Ala) produced in rice plants (genus *Oryzae)* appear to be related to the nature of the rice strain (Manabe, 1992). About 7% of the total alanine content in grass leaves consisted of D-Ala (Frahn et al., 1975). Exogenously supplied D-Ala, possibly of microbial origin, is required for the plant to synthesize the dipeptides. The biosynthetic pathways leading to the formation of these compounds and their functions in plants are not known.

Microbiology of D-Amino Acids. Microorganisms produce, metabolize, and utilize D-amino acids (Hopkins et al., 1997; Tunica et al., 1996). Because microbial D-amino acids enter the food chain, I will briefly examine the microbiology of D-amino acids that may be relevant to food science.

Peptidoglycans in Bacterial Cell Walls. Because constituents of bacterial cell walls may constitute a major source of dietary D-amino acids**,** understanding of the chemistry and biochemistry of peptidoglycans should contribute to the store of information concerning our knowledge about the composition and concentrations of dietary D-amino acids. The polymers making up the cell walls of Gram-positive (retain the stain of violet-iodine dye) and Gram-negative (do not retain the dye) bacteria differ significantly (Schleifer and Kandler, 1972; Zubai, 1983). The thick cell wall of a Gram-positive bacterium consists of peptidoglycan (a sugar−amino acid polymer) and teichoic acid (a polyol phosphate polymer). Both polymers contain D-amino acids. The much thinner cell wall of a Gram-negative bacterium consists entirely of peptidoglycan and associated proteins. The composition of the peptidoglycans and teichoic acids may vary among different classes of bacteria. The components of peptidoglycan include the amino sugars *N*-acetylglucosamine and *N*-acetylmuramic acid, cross-linked by branched-chain polypeptides containing D-amino acids, as shown in Figure 7. Penicillin and other antibiotics inhibit the illustrated transpeptidase-catalyzed cross-linking reaction between a Gly residue of one chain and a D-Ala residue of another, resulting in the elimination of a D-Ala residue in the last stage of the complex biosynthesis of the peptidoglycan. The D-amino acids in the bacterial cell walls contribute to their resistance to digestion by proteolytic enzymes.

In related relevant studies it was shown that (a) D-Ala is required for the synthesis of the mucopeptide component of nearly all bacterial cell walls (Thompson et al., 1998); (b) the peptidoglycan structure of *Lactobacillus casei* contained asparagine cross-links between D-Ala and L-Lys (Billot-Klein et al., 1997); (c) glutamate racemase in *Escherichia* coli provides D-glutamate, an indispensable component of peptidoglycans in bacteria (Liu et al., 1998a); (d) mutations leading to increased resistance of *Enterococcus faecalis* to antibiotics are governed by the amount of D-Ala-D-Ala and D-Ala-D-lactate incorporated into peptidoglycan precursors (Baptista et al., 1997); (e) the resistance to glycopeptide antibiotics in *Enterococci* results from the synthesis of peptidoglycan precursors with low affinity for these antibiotics (Reynolds, 1998); D−D-peptidase-catalyzed hydrolysis of the peptide bond in D-Ala-D-Ala seems to contribute to the resistance; (f) the role of D-Ala-D-Ala in enhancing and reducing microbial resistance to structural changes in vancomycin is described by Ge et al. (1999) and Sng et al. (1998); (g) the peptidoglycan and lipoteichoic acid from *Staphylococcus aureus* act synergistically in causing systemic inflammation and multiple system failure associated with Gram-positive bacteria (De Kimpe and Kengatharan, 1995); (h) the gene encoding D-threonine aldolase (an enzyme that catalyzes the inversion of D-Thr to D-*allo*-Thr in *E. coli*), the gene responsible for the incorporation of D-Ala into cell wall teichoic acid in *Bacillus subtilis* (Liu et al., 1998; Perego et al., 1995), and the genes of D-amino acid transaminase and glutamate of *Bacillus sphaericus* (Fotheringham et al., 1998) were cloned; (i) D-serine dehydratase from *E. coli* catalyzes the conversion of D-Ser, D-Thr, and *allo*-D-Thr to the corresponding α-keto acids and ammonia (Marceau et al., 1988); (j) the microorganism *Amycolatopsis* produces a thermostable bacterial *N*-acylamino acid racemase (Tokuyuma and Hatano (1995); (k) D-Ser inhibits the action of bacterial transaminase (aminotransferase), whereas D-Ala partially protects against this inhibition (Martinez del Pozo et al., 1989); and (l) D-amino acids inhibit the enzyme cytosolic serine hydroxymethyltransferase from *Euglena gracilis* (Sakamoto et al., 1993). The cited studies suggest that an understanding of the biochemistry of genes and enzymes involved in the synthesis and

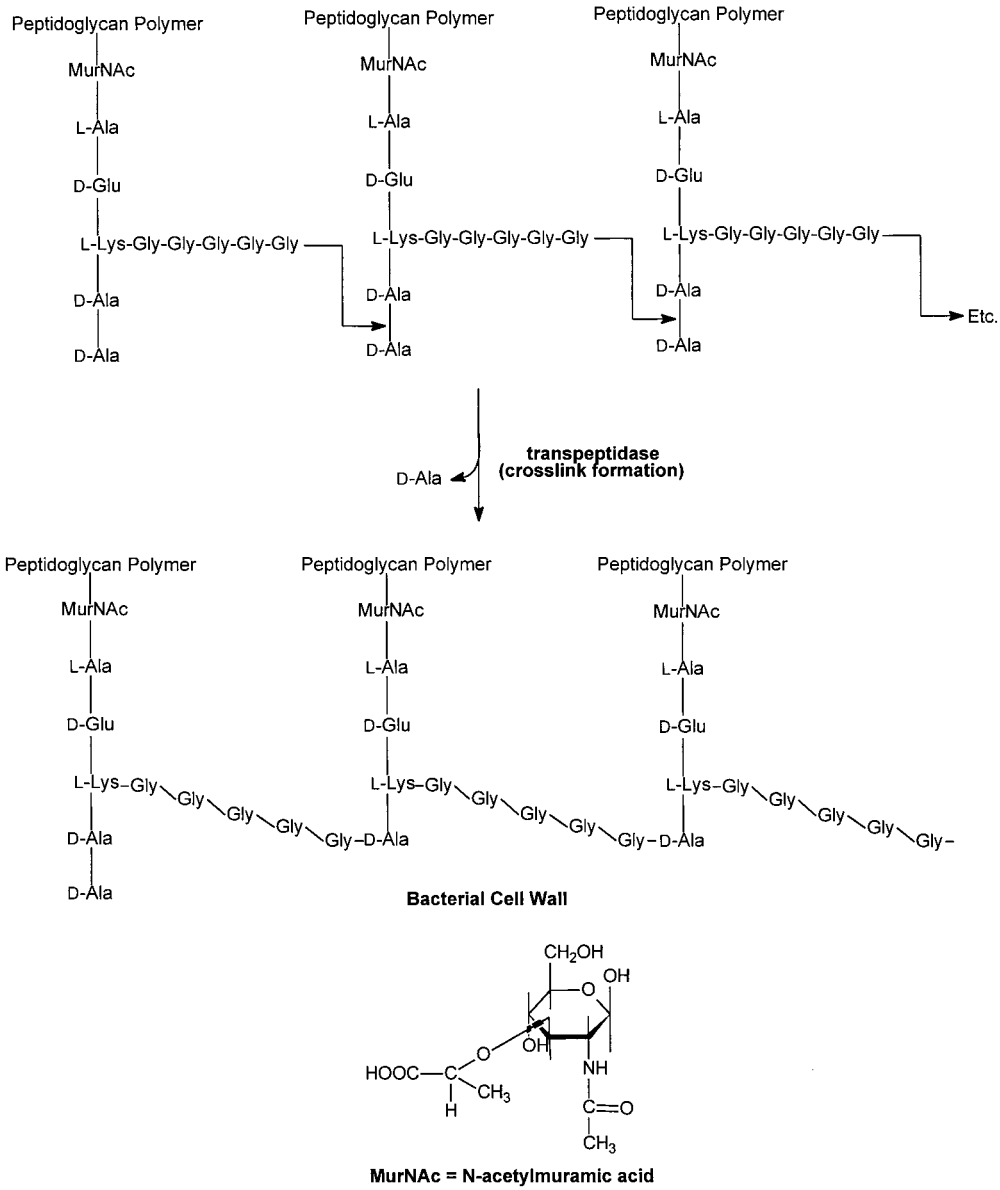

Figure 7. Final stage of peptidoglycan synthesis in the cell wall of *Staphylococcus aureus* showing transpeptidase-catalyzed cross-linking resulting from the displacement of a terminal D-Ala in one polymer unit by the terminal glycine residue of another [modified from Zubai (1983); see also van Heijenoort (1998)].

metabolism of D-amino acid-containing functional and structural microbial molecules which are targets of antibiotics may help efforts designed to discover improved bacteriocidal agents.

Peptide Antibiotics. D-Amino acid-containing synthetic and natural peptides possess strong antimicrobial properties. These include glycyl-D-Ala, myristoyl-D-Asp, sorbyl-D-Ala, and sorbyl-D-Trp, which appear to be effective against *Clostridium botulinum* (Paquet and Rayman, 1987), and natural antibiotics such as gramicidin (Mignongna et al., 1998; Ovchinnikov and Ivanov, 1982; Pollegioni et al., 1997b; Sahl and Bierbaum, 1998).

Certain antibiotics produced by bacteria and fungi contain D-amino acids that could enter the diet. These include D-Asp and D-Glu (bacitracin, mycobacillin), D-Cys (malformin), D-Leu (circulin), D-Orn (bacitracin), D-Phe (funigsporin, polymixin, tyrocidine), and D-Val (actinomycin and valinomycin) (Jack and Jung, 1998; Zubai, 1983).

The mechanism of action of peptide antibiotics containing D-amino acids appears to involve the disruption of bacterial cell membranes. To better define this aspect of antibiosis, Wade et al. (1990) prepared and evaluated three naturally occurring antibiotics, cecropin A, magainin 2 amide, and melitonin, in which they replaced all L-amino acids with D-enantiomers. Because the effectiveness of the L-isomeric antibiotics against *E. coli*, *Pediococcus auruginosa*, *B. subtilis*, *S. aureus*, and *Streptococcus pyrogenes* were 0.7−2 times greater than that of the corresponding D-isomers, both isomers appear to act by inducing ion channels in the lipid bilayers of the cell membranes. Such disruption requires no chiral selectivity. Because the D-isomers are expected to be more stable in vivo because of resistance to proteolytic digestion, they may be better candidates for use in animal and human infections. A similar mechanism probably operates during the antimicrobial action of potato glycoalkaloids against *S. typhimurium* (Friedman et al., 1997; Gubarev et al., 1998).

It would be interesting if normal proteins and peptides acquired antibiotic competence upon racemization.

Source of Bacterial D-Amino Acids. As mentioned

above, in addition to food processing, microorganisms and some of their products provide a potential source of dietary D-amino acids. To better define this aspect of human nutrition, Brückner et al. (1993) examined the D-amino acid content produced by several classes of bacteria (*Acetobacter*, *Bifidobacterium*, *Brevibacterium*, *Lactobacillus*, *Micrococcus*, *Propionibacterium*, and *Streptococcus*) used in starter cultures for the production of fermented foods and beverages. D-Ala and D-Asp were found at the highest concentrations in all bacteria. D-Glu was present in several classes of the microorganisms. Lower but significant amounts of D-Leu, D-Lys, D-Met, D-Orn, D-Phe, D-Pro, D-Ser, D-Thr, and D-Tyr were also detected in some of the bacteria. The results suggest that free D-amino acids in fermented foods, the latter comprising about one-third of the Western human diet, may largely originate from bacteria. Exposure of some of these foods to heat and high pH could generate additional free as well as protein-bound D-amino acids.

Bacteria from oral and intestinal floras and rumen microorganisms are also potential sources of D-amino acids present in body fluids and tissues (Brückner et al., 1992; Rooke et al., 1984). Feces must be a rich source of D-amino acids entering the aquatic and soil environments.

Enzymes from microbiological sources are finding numerous industrial applications as exemplified by the following examples: (a) α-keto acids can be stereospecifically converted to D-amino acids by a four-enzyme reaction system (Galkin et al., 1997); and (b) D- and DL-amino acids can be readily transformed to the corresponding α-keto acids by immobilized D-amino acid oxidase from *Rhodotorula gracilis* (Buto et al., 1994). This approach is useful for the transformation of the branched amino acids Ile and Val into the keto analogues, which are used therapeutically in the treatment of chronic uremias.

Fungi. Species of the fungi *Monascus* are used in Japan for preparing fermented food, for preserving meat, and as a source of the food colorants monascus color and monascus yellow. Sato et al. (1997) isolated and characterized eight such pigments, all of which contained both L- and D-amino acids. It is not known whether the D-amino acids occur naturally or arise during the manufacture of the pigments.

Insects. Insects are also reported to contain D-amino acids (Anand and Anand, 1994; Corrigan and Srinivasan, 1966). Insects can therefore serve as another source of D-amino acids for animals and humans. However, dietary D-Ala and D-Ser elicited adverse nutritional effects in insects and silkworms (Ito and Inokuchi, 1981). See also the section below on Nutrition and Safety.

Marine Invertebrates. Measurement of total D-amino acids using a D-amino acid oxidase technique revealed that the total tissue concentration of 18 species of marine invertebrates ranged from 0.04 to 44 mM (Okuma et al., 1998; Preston, 1987). The D-amino acids may originate from seawater, which contains significant amounts of L- and D-amino acids. The D-amino acids in the marine species could be involved in osmoregulation and serve as a nutritional source of L-isomers. The latter possibility is intriguing because, as noted below, the nutritional utilization of most D-amino acids is much slower than that of the corresponding L-isomers. Therefore, dietary D-amino acids in fluids and tissues could serve as a reservoir of L-isomers to be used in time of malnutrition and stress, when they can be used for the synthesis of structural and functional proteins needed for the survival of the organism.

The presence of D-amino acids containing peptides in frogs and molluscs arises from racemase-catalyzed transformation of L-amino acids to D-isomers (Fujita et al., 1997; Kreil, 1994a,b; Yasuda-Kamitani et al., 1995, 1997).

Rodents. Because D-Ser may modulate brain function by serving as ligand for the *N*-methyl-D-aspartate receptor in the forebrain, extensive efforts have been made to define its origin and function, especially its possible role in neurotransmission (Imai et al., 1997; Schell et al., 1995). High concentrations of D-Ser are present in the forebrains of mice, rats, and bulls (Armstrong et al., 1993a; Nagata et al., 1992, 1994a,b, 1995).

The free D-Ser in normal human brain tissue (D/L ratio = 0.086) was comparable to that in Alzheimer's-affected human brain (D/L ratio = 0.099). Because it (like the L-isomer) passes through the blood−brain barrier, D-Ser in the brain probably originates from the diet. D-Ser is metabolized by D-amino oxidase in the brain. In contrast, studies by Dunlop and Neidle (1997) suggest that synthesis of D-Ser in the brain by racemization of L-Ser, not dietary D-Ser, can account for the turnover of the brain D-Ser pool. These conflicting suggestions need to be resolved in view of the fact that although at high pH protein-bound L-Ser racemizes more rapidly than any other amino acid (Table 2), the amount of D-Ser in food proteins that have not been exposed to alkaline conditions appears to be too low to account for the relatively high amounts detectable in the brain.

The following is a brief summary of continuing studies on the role of D-amino acids in rodents. D-Amino acids, especially D-Glu, behaved as immunosuppressive agents in mice (Inoue et al., 1981). Mutant mice lacking D-amino acid oxidase activity contained 5.7 times more total Ser in the urine than normal mice (Asakura and Konno, 1997). The urinary D-Ser content (derived mostly from the diet) was 4.6 times greater in mutant than in normal mice. Studies on the distribution of radioactivities in rats following intravenous administration of ^{14}C-D- or -L-Ser showed that the highest concentrations of D-Ser were in the pancreas (12.6 nmol/g of wet tissue) and the kidneys (Imai et al., 1998). The high kidney levels could be responsible for the acute kidney necrosis induced by D-Ser, discussed below in the section on Nutrition and Safety. The possible role of D-Ser in the pancreas is not known.

Humans. *D-Amino Acids as Indicators of Aging.* There appears to be a direct relationship between the age of the collagen proteins in rat teeth and their D/L-Asp or D/L-hydroxyproline ratios, presumably because of little or no metabolic activity in the molar teeth after their formation (Ohtani, 1995; Ohtaini et al., 1995). The extent of Asp racemization can also be used to estimate the age at death (Lubec et al., 1994; Masters, 1986; Ohtani et al., 1998; Ritz, 1994). The use of spontaneous amino acid racemization of L- to D-Asp and of other amino acids to assess the age of tissues such as dentin, bone, and eye lens (Johnson and Miller, 1997; Man and Bada, 1987; Masters et al., 1978; Murray-Wallace and Kimber, 1993; Poinar et al., 1996) has been questioned by Clarke and colleagues (Brunauer and Clark, 1986; Geieger and Clark, 1987). Their objection is based on their findings that red cell protein aspartyl/asparagine residues racemize much more slowly in erythrocyte and other proteins than in proteins of the lens, tooth enamel,

and dentin. The in vivo rates of D-Asp formation in erythrocyte proteins were similar to the in vitro rates. It is not known whether biochemical processes exist to degrade or repair deamidated and isomerized aspartyl and asparagine residues in tissue proteins.

Additional factors that may complicate the use of amino acid racemization to estimate the aging process include the pH and temperature of the environment in addition to microbially induced promotion of amino acid racemization (Child et al., 1993).

Role of D-Amino Acids in Disease. D-Amino acids are present in normal and Alzheimer's-afflicted human brain, inhibit tumor growth in rats, and may be useful in cancer gene therapy (D'Aniello et al., 1992; Stegman et al., 1998). Although human plasma contained high concentrations of D-Ser, D-Ala, and D-Pro (D/L = 0.24, 0.21, and 0.1, respectively), no D-amino acids were present in plasma proteins (Huang et al., 1998; Nagata et al., 1992). The total D-amino acid content of the plasma correlated with the serum creatinine levels of the human subjects.

The total content of D-Phe and D-Tyr was significantly greater in patients suffering from chronic renal failure than in normal humans (Young et al., 1994). The increase in D-amino acids in renal failure may arise from preferential retention of D-amino acids in the kidneys (low glomerular filtration compared to L-isomers), depletion and inhibition of D-amino acid oxidase activity, and consumption of D-amino acid-containing foods and antibiotics. High D-Ser and D-Pro are also reported to accumulate during renal failure (Brückner and Hausch, 1993).

Healthy young adults can invert about one-third of D-Phe to its L-isomer (Tokuhisa et al., 1981). D-Phe is reported to induce analgesia in mice and humans (Balagot, 1983). The observed clinical relief in chronic pain patients given oral doses of 750−1000 mg of D-Phe is ascribed to inhibition of carboxypeptidase and the accompanying increase in the levels of brain met-enkephalin. Whether dietary levels of D-Phe present in processed food proteins can induce analogous pain relief in humans merits study.

Measurement of the stereochemistry of the lysine metabolite pipecolic acid, present in urine and plasma in humans suffering from inherited peroxisomal disorders, showed that the distribution of D- and L-pipecolic acids in the two body fluids in normal subjects differs from that in subjects with cerebrohepatorenal and related syndromes (Armstrong et al., 1993b). As is the case with other D-amino acids, the presence of D-pipecolic acid in plasma and urine appears to be generally diet related.

The previously mentioned involvement of D-Ser in neurotransmission is supported by a double-blind, placebo-controlled trial with 31 schizophrenic patients. This study revealed that D-Ser (30 mg/kg/day) added to an antipsychotic regimen had significant beneficial effects on cognitive function and performance (Tsai et al., 1998). The D-Ser was well tolerated by the patients with no apparent side effects. The authors suggest that D-Ser merits further evaluation in the treatment of schizophrenia. Will protein-bound D-Ser induce similar improvements?

Parenteral administration of D-Val to hepatoma-bearing rats induced significant improvement in nutritional status and tumor inhibition compared to control diets (Sasamura et al., 1998). The authors suggest that D-Val may be useful in the therapy of cancer.

The possible value of D-Tyr in treating hypertension (Segal, 1968) and the possible significance for human reproduction of the observed high content of D-Asp in rat testis fluids and tissues and spermatozoa (D'Aniello et al., 1998) merit sudy. It appears that D-amino acids possess both adverse and health-promoting attributes. The challenge is to optimize the beneficial ones.

NUTRITION AND SAFETY OF D-AMINO ACIDS

Two metabolic pathways are available for the biological utilization of D-amino acids (Kono and Yasumura, 1992): (a) epimerases or racemases may convert D-amino acids directly to L-isomers or to (DL) mixtures; or (b) amino acid oxidases may catalyze oxidative deamination of the α-amino group of amino acids to form α-keto acids, which can then be specifically reaminated to the L-form. Although both pathways may operate in microorganisms, until recently only the latter has been demonstrated in mammals (see D-Serine section below). The amounts and specificities of D-amino acid oxidases are known to vary in different animal species (Ramon et al., 1998; Wakayama et al., 1996). In some, the oxidase may be rate-limiting in the utilization a D-amino acid as a source of the L-isomer. In this case, the kinetics of transamination of D-enantiomers would be too slow to support optimal growth. These considerations, as well as differences in intestinal amino acid transport associated with stereoselectivity between D- and L-amino acids (Edelbroek et al., 1994; Scharrer, 1989; Schwass et al., 1983), largely explain the wide species dependence in the nutritional utilization of specific D-amino acids.

In addition, growth depression and cellular changes could result from nutritionally antagonistic and toxic manifestations of D-enantiomers exerting a metabolic burden on the organism. The situation is even more complicated for protein-bound D-amino acids, the nutritional effectiveness of which depends on the amino acid composition, digestibility, and physiological utilization of released amino acids. Because an amino acid must be liberated by digestion before nutritional assimilation can take place, the decreased susceptibility of D−D, D−L, and L−D peptide bonds in D-amino acid-containing proteins to digestion by proteolytic enzymes is a major factor adversely affecting the bioavailability of protein-bound D-amino acids. These aspects will be illustrated below with specific examples.

Digestibility of Racemized Casein. Friedman et al. (1981) attempted to quantitatively define the susceptibility of alkali-treated casein to in vitro digestion by trypsin and chymotrypsin. Monitoring on a pH-stat, we observed an approximately inverse relationship between D-amino acid and lysinoalanine content on one hand and the extent of proteolysis measured on the other (Figure 8). The findings were interpreted as follows: Trypsin catalyzes the hydrolysis of peptide bonds derived from carboxyl groups of Arg and Lys residues. Alkali transforms Lys residues to lysinoalanine side chains and simultaneously degrades Arg. The decreased susceptibility of alkali-treated casein to digestion could, therefore, arise partly from loss of susceptible sites to enzyme cleavage.

Chymotrypsin catalyzes the hydrolysis of peptide bonds of proteins adjacent to groups of aromatic amino acids (Phe, Tyr, Trp). The resistance of alkali-treated casein to hydrolysis by chymotrypsin may be due to

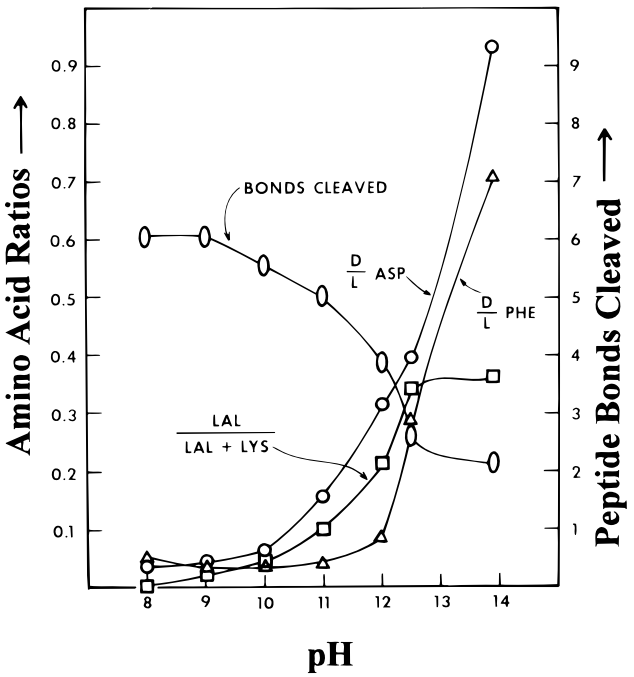

Figure 8. Inverse relationship between D-amino acid and lysinoalanine content of casein and extent of digestibility of peptide bonds by trypsin (Friedman et al., 1981). Reprinted with permission. Published 1981 by the Institute of Food Technologists.

racemization of aromatic side chains. The cited considerations are probably generally true for D-amino acid-containing food proteins (Bunjapamai et al., 1982; Hayashi and Kameda, 1980; Jenkins et al., 1984; Tovar and Schwass, 1983). As mentioned in the Introduction, the nutritional value of both unracemized and racemized proteins could be adversely affected by competition in the digestive tract for active sites of proteinases.

Biological Utilization and Safety of Specific D-Amino Acids. The following is a brief summary of nutritional and other health-related aspects of specific D-amino acids. This section complements the earlier discussion on the distribution of amino acids in different organisms. Although we are far from unraveling the intricate aspects of D-amino acid nutrition, the cited observations should facilitate the differentiation of beneficial and adverse effects following consumption of D-amino acids.

D-Methionine. The wide variation in the biological utilization of D-Met and D-Met analogues for growth of various animal species may be related to the activities of D-amino acid oxidases (Borg and Wahlstrom, 1989; Friedman and Gumbmann, 1984a,b; Gumbmann and Friedman 1986; Sarwar et al., 1989; Smalley et al., 1993; Figure 9A,D; Tables 3 and 4). However, the reported relative oxidation rates of D-Met by D-amino acid oxidase in kidney homogenates from man, monkeys, chicken, frogs, rats, and mice do not support this hypothesis (Konno and Yasamura, 1992). Thus, the reported rate of oxidation of D-Met by the oxidase in mouse kidneys is about one-third to one-half of the corresponding rates for rats or man. We have observed that the nutritional value of D-Met in mice is as good as that reported for either rats or man. In fact, D-Met appears to be poorly utilized by humans when consumed either orally or during total parenteral nutrition. One factor giving rise to inconsistencies in the utilization of

Table 3. Relative Potencies of L- and D-Methionine and Derivatives for Weight Gain in Mice Determined in All-Amino-Acid Diets (Friedman and Gumbmann, 1984a, 1988; Gumbmann and Friedman, 1988)

compound	relative potency, %
L-methionine	100.0
D-methionine	87.5
N-acetyl-L-methionine	89.6
N-acetyl-D-methionine	22.9
L-methionyl-L-methionine	99.2
L-methionyl-D-methionine	102.9
D-methionyl-L-methionine	80.4
D-methionyl-D-methionine	41.0
L-methionine sulfoxide	87.5
D-methionine sulfoxide	26.8
N-acetyl-L-methionine sulfoxide	58.7
N-acetyl-D-methionine sulfoxide	1.7
L-methionine hydroxy analogue	55.4
D-methionine hydroxy analogue	85.7

Table 4. Relative Growth Response to D-Amino Acids and Lysine and Histidine Derivatives in Mice Fed All-Amino-Acid Diets (Friedman and Finot, 1990; Friedman and Gumbmann, 1979, 1981; Friedman et al., 1982)

	potency relative to L-form, %
D-Met	79.5
D-Phe	51.6
D-Trp	24.7
D-Leu	12.4
D-His	8.5
1- or 3-*N*-methyl-L-His	0
D-Val	5.1
D-Thr	3.1
D-Ile	1.2
D-Lys	−4.4, −11.8[a]
L,L + D,L-lysinoalanine	3.8
N-ε-benzylidene-L-lysine	85.4
N-ε-(γ-glutamyl)-L-lysine	51.1
N-ε-acetyl-L-lysine	40.0
N-ε-formyl-L-lysine	40.0
N-α,*N*-ε-diformyl-L-lysine	16.8
N-ε-methyl-L-lysine	12.3
N-ε-dimethyl-L-lysine	9.4
N-ε-trimethyl-L-lysine	8.2

[a] Values from two separate experiments. Negative signs indicate weight loss relative to the control diet lacking L-Lys.

D-Met is dose dependency of the apparent potency of D-Met relative to its L-isomer, that is, the dietary level of the D-form for any given growth response relative to that of the L-form which would produce the same growth response. This dose dependency is a result of the nonlinear nature of the dose−response curves. This complicates attempts to compare results from some studies with other animal species, which often report data based on a single substitution of the D- for the L-isomer.

The extent of utilization of D-Met (and other D-amino acids) by D-amino acid oxidases may not always be the limiting step governing utilization. Other factors that could influence utilization include rate of intestinal transport; action of intestinal enzymes and bacteria; rates of absorption, renal clearance, and possible toxic effects of excess L-Met. Figure 9A shows that consumption of high levels of L-Met depresses weight gain in mice, whereas D-Met does not.

Selenomethionine Isomers. Although excess consumption of selenium is undoubtedly toxic, especially to aquatic birds, small amounts may protect against cancer, presumably because of the anti free radical action of the selenium-containing enzyme glutathione

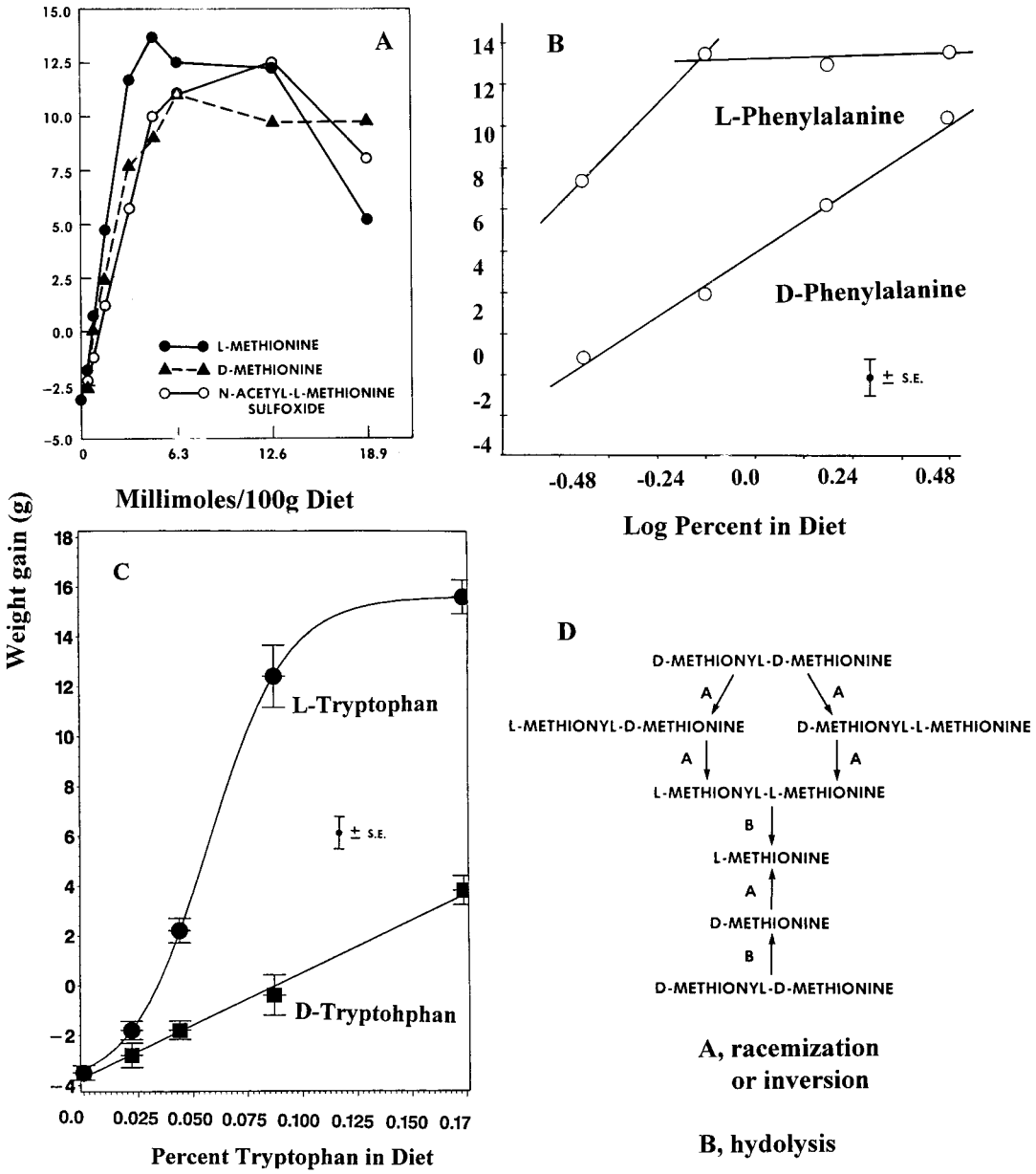

Figure 9. Relationships of weight gains to percent of L- and D-amino isomers in amino acid diets fed to mice: (A) Met isomers; (B) Phe isomers; (C) Trp isomers; (D) possible pathways for the in vivo transformation of D-methionyl-D-methionine (Friedman, 1988; Friedman and Gumbmann, 1984a,b). Reprinted with permission. Published 1984 and 1988 by the American Institute of Nutrition (A, B, D).

peroxidase. Because selenomethionine in the diet may be subject to food processing conditions before consumption, Boehm and Bada (1985) determined the racemization rate of L-selenomethionine present in selenium-rich yeast at pH 7.4 and 100 °C. The time required to reach a D/L ratio of 0.33 (racemization half-life) of 19−20 days was similar to that observed with Met. These observations suggest that very little racemization of free Met or selenomethionine will occur under normal cooking conditions.

Because selenomethionine is a major source of selenium in the diets of both animals and humans, the question of relative biopotencies of selenomethionine isomers arises. Comparison of the effects of oral consumption of seleno-L-methionine, seleno-DL-methionine, and selenized yeast on the reproduction of mallard ducklings revealed that although both selenomethionine preparations were of similar toxicity, their potency was greater than that of selenium present in yeast (Heinz and Hoffman, 1996). In a related study, Heinz et al. (1996) found that the survival of day-old ducklings consuming L-selenomethionine after 2 weeks was significantly lower (36%) than that of ducklings consuming the DL-isomer (100%). The greater lethality induced by the L-isomer may be due to differences in palatability or the nutritional nature of the wheat-based diets used. Because of possible antagonistic or synergistic behavior of the two isomers in the DL-mixture used, it would be of interest to find out the relative potency of pure D-selenomethionine.

D-Cystine. In a discussion of D-cystine, it is informative to first ascertain the utilization of the L-isomer. Although L-cystine is not an essential amino acid for rodents, less L-methionine is needed for growth if the diet contains L-cystine (Friedman and Gumbmann, 1984a). The mechanism of this so-called sparing effect needs to be clarified. One possibility is that L-cystine

serves as a source of L-cysteine, thus minimizing the need for L-Met to serve as a reservoir for these amino acids.

In addition to racemization, exposure of cystine residues in proteins (P−S−S−P) to high pH can cleave disulfide bonds, leading to the formation of a variety of oxidized and desulfurized products such as P−S−SO−P, P−SO−SO−P, and P−SO_3H. The chemistry and nutritional signficance of these transformations are discussed elsewhere (Friedman, 1973, 1994, 1999).

Our results show that L-cystine is somewhat more efficient in sparing D-Met than L-Met in amino acid diets containing low levels (0.29%) of the two isomers. Supplementation of D-Met with an equal sulfur equivalent of L-cystine nearly doubled growth, bringing the overall response equal to that produced by L-Met in the presence of L-cystine. These results demonstrate a lessening in demand for L-Met when either L- or D-Met is available. In contrast, supplementation of suboptimal levels of L-Met with increasing concentrations of D-cystine reduced the growth rate of mice, which may imply that excess D-cystine in the diet is toxic.

D-Cysteine. Although L-cysteine had a sparing effect of L-Met consumed by mice, D-cysteine did not (Friedman and Gumbmann, 1984a). In fact, D-cysteine imposed a metabolic burden as indicated by depressed growth when fed to mice with less than optimal levels of D-Met. The 24% decrease in weight gain of the D-cysteine plus L-Met amino acid diet compared to L-Me alone implies that D-cysteine is nutritionally antagonistic or toxic. The mechanism of this effect is not known.

Lanthionine Isomers. Lanthionine isomers (Figure 1B) are formed naturally during the biosynthesis of microbially derived peptide antibiotics (Sahl and Bierbaum, 1998) and during exposure of proteins to alkali and heat (Friedman, 1999). Such treatments generate dehydroalanine side chains from cystine and serine. Reaction of the SH group of cysteine and the double bond of dehydroalanine gives rise to one pair of optically active D- and L-isomers and one diastereomeric (*meso*) form of lanthionine (Friedman and Noma, 1979; Küsters et al., 1984; Figure 1B). The mixture of DL + *meso*-lanthionine isomers has a moderate sparing effect on L-Met, as evidenced by a 27% greater weight gain when the two amino acids were fed together than when suboptimal L-Met was fed (Friedman and Gumbmann, 1984a, 1988). The biological utilization of the individual isomers merits study.

D-Phenylalanine. Our studies revealed that the relative growth of mice fed D-Phe replacing the L-isomer in an all-amino-acid diet is concentration-dependent, ranging from 28.3 to 81.3% when compared to control diets containing the same amounts of L-Phe (Figure 9B; Friedman and Gumbmann, 1984b). The data suggest the absence of any antinutritional effects or toxicity from feeding either Phe isomer at twice the optimum dietary level.

It might also be worthwhile to speculate about the possible effect of D-Phe on phenylketonuria in children (Anonymous, 1983). Figure 9B shows that the D-isomer, because it must be first inverted, is utilized more slowly than L-Phe; therefore, it might not have the same effect on phenylketonuria as the L-isomer. The slow and uniform release of L-Phe from D-Phe observed in mice could possibly provide a basis for the utilization of D-Phe in the treatment of phenylketonuria. Animal and human studies are needed to demonstrate this possibility.

Exposure of the artificial sweetener aspartame (L-aspartyl-L-phenylalanine methyl ester) at 100 °C at neutral pH results in the formation of a diketopiperazine and the racemization of both constituent amino acids (Boehm and Bada, 1984). Because pure D-amino acids are generally sweet compared to the bitter taste of L-isomers (Solms, 1969), it is not known whether the observed racemization of the peptide-bound amino acids affects taste.

D-Tyrosine. Nutritionally, L-Tyr is classified as a semiessential amino acid (Mercer et al., 1989). Combinations of L-Tyr and L-Phe are complementary in supporting the growth of mice (Friedman and Gumbmann, 1984b). Thus, under conditions where L-Phe may be limiting, L-Tyr may supply half the requirement of L-Phe alone for mice, a value similar to those reported for humans, chicks, and rats.

Our feeding studies also revealed that with D-Tyr in an amino acid diet, growth inhibition was severe at a D-Tyr/L-Tyr ratio of 2:1 but was much more moderate when the ratio was 1:1. Similar results were obtained with a casein diet supplemented with D-Tyr. The antimetabolic manifestation of D-Tyr may be ascribed to interference with normal amino acid transport and the biosynthesis of vital neurotransmitters and proteins in vivo. One or more of the following mechanisms may explain the observed interference (Anonymous, 1985; Friedman and Gumbmann, 1984b).

Because D-Tyr was incorporated into tyrosyl-tRNA to form a tyrosyl-tRNA derivative similar to that formed by L-Tyr (Calendar and Berg, 1967; Yamane et al., 1981), incorporation of D-Tyr will lead to the formation of faulty proteins. A related possibility is the suppression of normal protein synthesis by D-Tyr through competitive inhibition of incorporation of L-Tyr or L-Phe into aminoacyl-tRNA. D-Tyr could also interfere in the biosynthesis or biological action of vital neurotransmitters such as dopamine. The hypotensive effect of D-Tyr is postulated to involve this effect (Segal, 1982). Other possibilities include interference by D-Tyr in the hydroxylation of L-Phe to L-Tyr and overloading metabolic pathways needed to eliminate or detoxify excess D-amino acids.

Our findings demonstrate that D-Tyr, unlike L-Tyr, has no sparing effect for L-Phe in mice. In fact, a metabolic stress, in the form of growth inhibition, may become evident when D-Tyr is present in the diet at molar concentrations equal to or greater than L-Phe. This acute effect remains to be defined toxicologically, and the potential for subchronic and chronic toxicity following exposure to lower levels of D-Tyr remains unknown. It is reassuring that by recycling D-Tyr-$tRNA^{Tyr}$ into free $tRNA^{Tyr}$, the widely distributed enzyme D-Tyr-$tRNA^{Tyr}$ deacylase protects cells against misincorporation of D-Tyr (Soutourina et al., 1999).

D-Tryptophan. The nutritionally essential amino acid L-Trp is exceptional in its diversity of biological functions. It contributes to protein synthesis and regulates numerous physiological mechanisms. These include serving as a precursor of the neurotransmitter serotonin and of the vitamin niacin. The relative nutritional potency of D-Trp compared to that of the L-isomer in mice is strongly dose-dependent, being inversely related to the dietary concentration and ranging from 29 to 64% (Figure 9C; Friedman and Cuq, 1988). The maximum

growth obtainable for L-Trp occurred at 0.174% in the diet. By increasing the dietary concentration of D-Trp up to 0.52%, it was possible to demonstrate for D-Trp that growth also passed through a maximum at 82% of that achieved with the L-isomer. This occurred at 0.44% of D-Trp in the amino acid diet.

Considerable species variation is known to exist for the nutritive value of D-Trp (Borg and Wahlstrom, 1989). In chicks fed amino acid diets, the relative potency of the D- to the L-isomer has been reported to be 20%. The corresponding value for humans is ~10%. In contrast, rats seem to utilize D-Trp efficiently as a nutritional source of L-Trp. Although possible reasons for this variation need to be better defined, D- and L-Trp exhibit stereospecific effects on pyloric duodenal motility and gastric emptying in animals and humans (Edelbroek et al., 1994).

D-Lysine and Lysine Derivatives. Exposure of sunflower protein to 0.2 N NaOH at 80 °C induced the formation of D-Lys (Provansal et al., 1975). D-Lys is not utilized as a nutritional source of L-Lys by chicks, dogs mice, rats, or humans, presumably because D-amino oxidase does not act on D-lysine. The amino acid lysinoalanine, formed concurrently with D-amino acids in alkali-treated proteins, has two asymmetric C atoms, making possible four separate diastereoisomeric forms: DD, DL, LD, and LL. The nutritional value of the individual LAL isomers as a source of L-Lys is not known. Table 4 shows that a mixture of LL and LD isomers has a nutritional value for the mouse equivalent on a molar basis to 3.8% of L-Lys. For comparison, the table also lists the nutritional values of several other lysine derivatives as well as D-amino acids we determined. The four lysinoalanine isomers differ in their ability to chelate metal ions such as copper (Friedman and Pearce, 1989).

Because L-Lys is a nutritionally limiting amino acid in cereal proteins such as wheat gluten, the transformation of even a small fraction of L-Lys to the D-isomer and to lysinoalanine is expected to adversely affect the nutritional quality of cereal proteins to a greater extent than would be the case for legume (e.g., soy) and animal (e.g., casein) proteins, the L-Lys content of which is much higher (Friedman, 1996a,b, 1997; Friedman and Finot, 1990; Friedman et al., 1991; Smith and Friedman, 1984).

D-Serine. Although L-Ser is not an essential amino acid, its presence in the diet is of interest because this D-amino acid is reported to induce enlargement of rat kidney cells (cytomegaly) similar to that observed with lysinoalanine (Carone et al., 1985; Kaltenbach et al., 1979). The accumulation of intravenously administered ^{14}C-D-serine in the kidney suggests a possible link between acute necrosis of renal proximal tubules and D-Ser (Imai et al., 1998). This conclusion is supported by the reabsorption of D-Ser in the rat renal pars recta (Silbernagl et al., 1999). Whether the localization and reabsorption of D-Ser in the renal pars recta also take place in humans merits study.

D-Ser also may also involved in neurotransmission in the brain (Kapoor and Kapoor, 1997) and, as mentioned earlier, was beneficial in the treatment of schizophrenia. The cerebral cortex of the rat has the ability to transform L-Ser to D-Ser by a mechanism involving racemases similar to that operating in bacteria (Wolosker et al., 1999).

The toxicity of D-Ser, D-Cys, and D-Pro may arise from oxidative damage to cells induced byproducts of their metabolism such as H_2O_2 (Ercal et al., 1996). It is also worth noting that protein-bound L-Ser racemizes more rapidly to the L-isomer than any of the other amino acids (Table 2; Friedman and Liardon, 1988). See also the above sections on Rodents and Humans.

D-Threonine. The essential amino acid L-Thr is second-limiting in corn proteins. The utilization of D-Thr by the chick, rat, mouse, or human as a nutritional source of the L-isomer is insignificant (Borg and Wahlstorm, 1989). As mentioned earlier, Thr has two asymmetric C atoms; four diastereoisomeric forms are therefore possible. The biological and nutritional significance of the individual isomers merits study.

D-Proline. L-Pro is a nonessential amino acid. Oral feeding of an aqueous solution of D-Pro (50 mg/kg of body weight/day) for 1 month to rats induced fibrosis and necrosis of kidney liver cells and elevation of serum enzymes (Kampel et al., 1990). No D-Pro was detected in the serum, liver, kidney, or urine of the dosed rats. However, these observations could not be confirmed by Schieber et al. (1997), who found no hepatotoxic or nephrotoxic manifestations in rats following oral consumption of the same amounts of D-Pro or D-Asp solutions for 28 days. There was a 20−30-fold increased renal excretion of D-Pro in rats on the test diets compared to controls. These conflicting results need to be reconciled.

Pro is the most abundant free amino acid in wine. Calabrese et al. (1995) analyzed wines of different ages and found that L-Pro is present only in young wines and that D-Pro appears in wines that are ~5 years old. The content increased with age, reaching a maximum of ~15% D after 30 years. Isomerization of L- to D-Pro in vinegar and wine has been attributed to acid-catalyzed racemization. This is unlikely, in view of the fact that the D-forms of other rapidly racemizing amino acid such as Asp and Ser were not observed in vinegars. A more likely possibility is the occurrence of microbiological enzymatic racemization (Erbe and Brückner, 1998). The D-Pro content of vinegars and wines and possibly other alcoholic beverages could serve as a rough indicator of their ages (Armstrong et al., 1990; Chiavaro et al., 1998). Our studies showed very low racemization of Pro in soy proteins exposed to high pH (Friedman and Liardon, 1985). Low levels of D-Pro in honey (Pawlowska and Armstrong, 1994) and the presence of added D-Pro in fruit juices (Kuneman et al., 1988) can be readily detected.

Other Amino Acids. According to Borg and Wahlstrom (1989), the biological utilization of the following D-amino acids by different animal species and humans needs to be better defined: Arg, His, Leu, Ile, and Val (see Table 4). For these and other amino acids, a need exists to standardize methods designed to ascertain the role of D-amino acids in nutrition. Supplementation of proteins with D-amino acids may not always be a reliable approach to solving this problem. The use of all-amino-acid diets in which the test amino acid (L-isomer) is completely replaced with different levels of the corresponding D-amino acid may be preferable, because the organism is then forced to use the D-amino acid as the sole source of the L-form. However, this approach may not always predict the utilization of protein-bound D-amino acids for two reasons: (a) as discussed above, D-amino acids along a peptide chain may be less digestible than the L-forms; and (b) the utilization of

any D-amino acid appears to be affected by the presence of other D-amino acids in the protein. All of them need and thus compete for the available D-amino acid oxidase (D'Aniello et al., 1993), the synthesis of which may depend on the dietary protein content of the liver (Carreira et al., 1996).

RESEARCH NEEDS

The described results and interpretations should contribute to our understanding of the origin and nutritional, microbiological, physiological, pharmacological, and medical consequences of ingesting naturally occurring and processing-induced D-amino acids. Unresolved is whether the biological effects of D-amino acids vary, depending on whether they are consumed in the free state or as part of a food protein. The following aspects also merit further study: whether nonaqueous solvents such as dimethyl sulfoxide, which are known to influence reaction rates of protein functional groups (Friedman, 1967; Friedman and Williams, 1977; Friedman et al., 1984b), will also change the kinetics of racemization; whether poorly digestible racemized food proteins, the conformations of which differ from those of the native proteins, could serve as dietary fiber in the digestive tracts of animals and humans; whether food-derived amino acids and peptides alter the normal microflora of the intestine; whether metabolic interaction, antagonism, or synergism among free and protein-bound D-amino acids occurs in vivo; whether racemization, known to alter protein conformation, might have protective effects against protein-induced allergy, celiac disease, and HIV (Huq et al., 1999) and other infections; and whether D-amino acids and peptides can competitively inhibit bacterial D-amino acid oxidases and D-transpeptidases and thus inhibit the biosynthesis of peptidoglycans in bacterial cell walls and the multiplication of human pathogens (Pouny and Shai, 1992; Sawyer et al., 1983; Shepherd et al., 1994; Sng et al., 1998; Toomik et al., 1994). Other active areas of research include assessments of possible beneficial effects of substituting D- for L-amino acids in biologically and pharmacologically active peptides and proteins (Balasubramaniam et al., 1994; Galantino et al., 1995), the study of enzyme mechanisms with substrates containing D-amino acid residues (Eller et al., 1993), and effects of high pH on phenolic compounds (Friedman and Jürgens, 1999) and other food ingredients. We are challenged to respond to these interrelated multidisciplinary research needs in the chemistry and biology of D-amino acids and related ones mentioned earlier.

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