Heat and Alkaline Damage to Proteins: Racemization and Lysinoalanine Formation

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Treatment of protein material at high temperatures or at high pH can cause racemization and cross-linking of amino acyl residues. Evidence suggesting that different proteins show varying susceptibilities toward these phenomena has been reported in the literature. A gas chromatographic method was used in the systemic investigation of racemization and lysinoalanine (LAL) formation in alfalfa leaf protein concentrate (LPC), lactalbumin (LA), and isolated soy protein (ISP), while varying the time, temperature, and pH of treatment. Results of this study indicate that differences in racemization between proteins do exist and that serine racemization is the most sensitive indicator of racemization among the amino acids which can be measured by this technique. Furthermore, LAL formation is greatest under conditions which are less harsh than those which induce the greatest racemization.

Severe heat or alkaline treatments can result in significant changes in the properties of proteins. Alkaline treatment of protein-containing foods is used to alter flavor or texture (Smith and Circle, 1971), to destroy toxins (Masri et al., 1969), enzymes (Andres, 1979), or inhibitors (Liener and Kakade, 1969; Badenhop and Hackler, 1970), and to promote solubilization of proteins (Fellers et al., 1966). Heat treatment is used for sterilization/pasturization (McCulloch, 1936; Douglas et al., 1981) and to promote desirable physical characteristics in the protein or food system (Circle et al., 1964; Potter, 1978). Less desirable chemical changes such as reduced bioavailability due to loss of lysine via Maillard reactions (Carpenter, 1973), isopeptide and cross-link formation (Ziegler, 1964), and racemization of amino acyl residues (Levene and Bass, 1928; Provansal et al., 1975; Asquith and Otterburn, 1977; Hayase et al., 1979) may also occur when proteins are subjected to heat and strongly alkaline conditions. In addition to the problem of reduced nutritional quality as a result of alkali or heat treatment, lysinoalanine (or a metabolite of this compound) and D-serine have been found to be nephrotoxic in rats (Fishman and Artom, 1942; Artom et al., 1945; deGroot and Slump, 1969; Provansal et al., 1975; Woodard and Short, 1977).

Reports have appeared that suggest that there is variation in susceptibility to cross-linking reactions among proteins (Hayashi and Kameda, 1980a, b; Haraguchi et al., 1980; Manson and Carolan, 1980; Hasegawa et al., 1981; Neiss, 1981). This implies that some high-protein products may be resistant to a particular heat/alkaline processing regimen while others may suffer significant chemical changes under the same conditions. Therefore, it is important that the factors affecting these changes be understood in order to control decreases in nutritional quality.

The purpose of this study was to document racemization and lysinoalanine (LAL) formation over a comprehensive range of temperature, time, and pH using purified proteins as model systems. The use of a gas chromatographic technique employing an optically active capillary column allowed extension of previous data to more amino acids. This report also describes how a single derivatization technique can be used for the measurement of both racemization and LAL formation. The proteins used in this study were alfalfa leaf protein concentrate (LPC), lactalbumin (LA), and isolated soy protein (ISP).

MATERIALS AND METHODS

Lactalbumin was purchased from United States Biochemical Corp. Cleveland, OH, and ISP (Promine D) was obtained from Central Soya, Chicago, Illinois. Alfalfa LPC was obtained from B. E. Knuckles at this laboratory. Pentafluoropropionic anhydride was purchased from Pierce Chemical Co., Rockford, IL. Amino acid isomers were purchased from Sigma Chemical Co., St. Louis, MO. Reaction vessels were $^1/_2$-dram vials purchased from Wheaton Scientific, Millville, NJ, and $^1/_{32}$-in. Teflon sheet stock for vial cap liners was purchased from Bolab, Inc., Derry, NH.

Treatment of Proteins. One percent suspensions of each protein were prepared by mixing (with minimal air incorporation) into aqueous buffered or sodium hydroxide solutions prior to heat treatment. The suspensions were held at pH 3.3 (0.1 M sodium formate buffer), pH 5.0 (0.1 M sodium acetate buffer), pH 7.0 (0.1 M sodium phosphate buffer), pH 8.5 (0.1 M disodium phosphate buffer), pH 10.0 (0.1 M sodium borate buffer), and pH 12.8 (0.1 N sodium hydroxide). Treatment temperatures between 22 and 100 °C were obtained by placing 25 mL of each sample in a loosely stoppered 50-mL Erlenmeyer flask in a water bath maintained to ±1 °C. For the samples held in 0.1 N sodium hydroxide, the incubations were terminated by neutralizing with hydrochloric acid (HCl). The other treatments were terminated by freezing in a dry ice–ethanol bath. The samples were then lyophilized before portions were taken for acid hydrolysis. Selected samples were resuspended in H_2O and dialyzed against 0.02 M acetic acid, pH 3.24, for 48 h before a second lyophilization and subsequent acid hydrolysis.

Detection of Optical Isomers and LAL. Samples were hydrolyzed in 6 N HCl (15 mg of sample/10 mL of HCl) at 110 °C for 24 h under less than 10 μm of pressure. The hydrolysate was evaporated and washed 3 times with deionized–distilled H_2O on a rotary evaporator to remove HCl. The hydrolysates were then redissolved in approximately 3 mL of deionized H_2O, quantitatively transferred to a 5-mL sample vial, dried under a stream of purified nitrogen at 50 °C, and dissolved in 1.00 mL of 80% ethanol, and 200 μL of the solution was transferred to a $^1/_2$-dram vial fitted with a Teflon-lined cap. The samples were then dried under a stream of purified N_2 at room tem-

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perature in preparation for derivatization to the *N*-pentafluoropropionyl, *O*,*S*-isopropyl amino acids using the basic procedure of MacKenzie and Tenaschuk (1974). Esterification was accomplished by reacting the samples with 600 μL of 3 N HCl in dried 2-propanol at 110 °C for 1 h. The esterified mixture was allowed to cool to room temperature before the solvent was evaporated under a stream of purified N_2. Acylation was accomplished by adding 350 μL of redistilled pentafluoropropionic anhydride to the vial, tightly sealing, and holding at 150 °C for 15 min. The sample was then cooled to room temperature and evaporated to dryness under a stream of purified N_2. The derivatized sample was then dissolved in 20 μL (for isomer analysis) or 500 μL (for LAL analysis) of distilled, anhydrous ethyl acetate.

Optical isomers of the amino acid derivatives were separated and quantitated by using a Hewlett-packard 5840 gas chromatograph equipped with an inlet stream splitter (1:100) and fitted with a Chirasil-Val glass capillary column (Applied Science Laboratories, Inc., State College, PA), 25 m × 0.3 mm. Helium was used as the carrier gas at a linear velocity of 25 cm·s^{-1}. The temperature program was 4 min at 90 °C followed by a rate of 4 °C·min^{-1} to a final temperature of 170 °C, which was held for 21 min. The injector was held at 170 °C, and the flame ionization detector was held at 225 °C. Retention times were identified by using synthetic D or L isomer derivatives, and percent D isomer was calculated as the area of the D isomer peak divided by the sum of the areas of the D and L isomer peaks: (area of D isomer/areas of D and L isomers) × 100. Racemization of isoleucine and threonine was not measured as the appearance of the D-allo forms of these amino acids.

Lysinoalanine determinations were performed on a Hewlett-Packard 5830 gas chromatograph equipped with a splitless injector and fitted with a WCOT glass capillary column, 25 m × 0.5 mm, coated with OV-17. Helium was used as the carrier gas at a linear velocity of 28 cm·s^{-1}. The temperature program was 1 min at 70 °C followed by a rate of 30 °C·min^{-1} for 3 min, 4 °C·min^{-1} for 7.5 min, 1 °C·min^{-1} for 6 min, and 30 °C·min^{-1} to a final temperature of 250 °C, which was held for 10 min. The injector was held at 225 °C and the flame ionization detector at 300 °C. Venting of the splitless injector occurred at 0.3 min. Retention times of LAL were determined by using derivatized material synthesized in this laboratory (Finley and Snow, 1977) and commercially obtained material (Miles, Research Products Division, Elkhart, IN).

The retention times of the LL and LD isomers of LAL were 15.50 and 15.75 min, although not necessarily in that order because we did not have standards that were resolved into isomers. These peak areas were combined when calculating LAL content. The detector response was linear (correllation coefficient = 0.968) with LAL mass to 1500 nmol·assay^{-1}, which was the largest amount tested (Schwass and Horn, 1982), and the largest sample values measured did not exceed 400 nmol·assay^{-1} [approximately 2.3 g of LAL·(100 g of protein)$^{-1}$]. Values obtained for LAL by using this method were within ±5% of the values measured by amino acid analysis.

RESULTS AND DISCUSSION

Effect of Temperature and Time on Racemization. The extent of racemization of several amino acids in LA, LPC, and ISP was investigated at several temperatures (22, 37, 55, 85, and 100 °C). Treatments were carried out for various lengths of time (30, 60, 120, or 240 min) in 0.1 N sodium hydroxide. The results of these experiments are shown in Figure 1. Isoleucine, proline, threonine, and valine residues showed very little tendency to racemize over the studied temperature range. Alkali-treated wheat gluten has been shown to have low racemization of proline and valine (isoleucine and threonine not measured) (Masters and Friedman, 1979), and alkali-treated casein shows the lowest racemization for these four amino acids (Liardon and Hurrell, 1983). Alanine, glutamic acid (plus glutamine), leucine, and lysine all exhibited relatively low racemization until the temperature exceeded 55 °C. Significant racemization (~30–45% present as the D isomer) occurred at 85 and 100 °C in samples held for 120 and 240 min. Aspartic acid (which includes asparagine because of

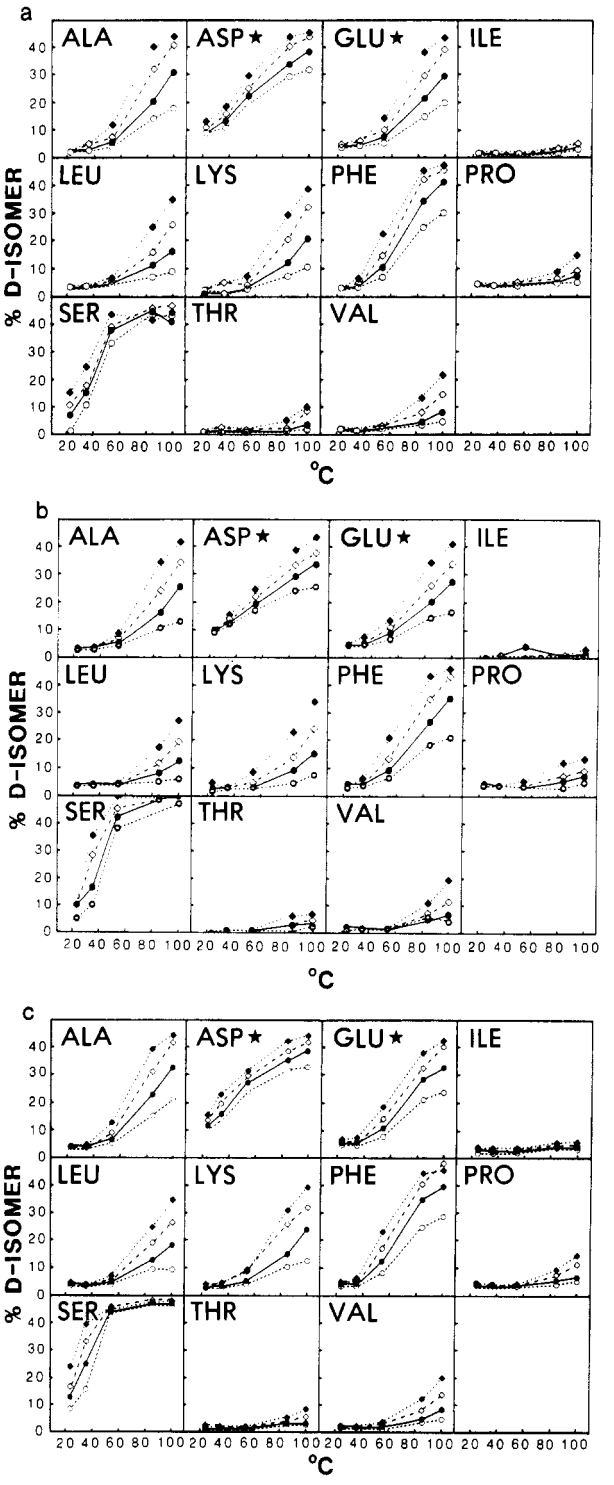

Figure 1. Effect of temperature on racemization of proteins held in 0.1 N sodium hydroxide for 30 (○), 60 (●), 120 (◊), or 240 min (♦). Panel a, alfalfa LPC; panel b, LA; panel c, ISP (★ indicates the combined value for the acid and amide).

the amide is hydrolyzed during acid hydrolysis of the protein) and phenylalanine showed a greater tendency to racemize at lower temperature, with aspartic acid–asparagine (Asp–Asn) racemization showing nearly linear temperature dependence from 22 °C. Serine stands out as being the most easily racemized residue under these conditions: racemization was essentially complete (50% present as the D isomer) in all three proteins after 60 min at 55 °C. Racemization of serine is much higher than for any other amino acid at any time at temperatures under 55 °C except Asp–Asn at 22 °C. The elevated values for Asp–Asn racemization at this low temperature are typical for most proteins and are probably due to acid-catalyzed racemization during the hydrolysis step (Liardon et al., 1981). Values for racemization in untreated proteins were similar to the values observed for the proteins held at 22 °C for 30 min (Schwass and Finley, 1981).

These results show that serine and aspartate are the residues that undergo the greatest racemization at the lowest temperatures, i.e., the least harsh conditions. While several investigators have demonstrated that aspartate is easily racemized under alkaline conditions (Bada, 1972; Masters and Friedman, 1979; Hayase et al., 1979; Neiss, 1981), this study and work of others shows that serine generally isomerizes to a greater extent when racemization of both residues is measured (Hill and Leach, 1964; Tovar, 1981; Bunjapamai et al., 1982; Liardon and Hurrell, 1983). This disparity may be due to the participation of the β-carboxyl group of aspartate in salt bridges within the protein that could cause lowering of the effective inductive strength of that carboxyl group under conditions where the protein is not denatured completely. Friedman et al., (1981) have suggested that aspartate inversion might be used as an index of racemization within a protein. The present results suggest that serine can provide a more sensitive indicator of racemization, although for proteins such as casein, where easily racemized phosphoserine is abundant (Hayashi and Kameda, 1980b), overly high estimates of racemization could result. A second situation where serine may not be a satisfactory indicator is where heat but not alkalinity has been used because serine is less racemized under these conditions. For example, Liardon and Hurrell (1983) have shown aspartate (in chicken muscle protein) and cysteine and aspartate (in bovine plasma albumin) to be approximately 2–6 times as racemized as serine under conditions of heating only. In the same study, serine was the most racemized amino acid when casein was treated with alkali and heat.

In general, for the amino acids measured, the degree of racemization of a given amino acid residue of a protein held at a constant temperature for a specified duration was quite similar among these proteins. These results are supported in part by the results of Masters and Friedman (1979), who investigated seven amino acids and reported similar rates of racemization among proteins, including soy isolate and lactalbumin. Although some investigators have shown differences in racemization between selected proteins or peptides (Pickering and Li, 1964; Lande and Landowne, 1966; Smith and deSol, 1980; Hayashi and Kameda, 1980b; Tovar, 1981), the pattern that seems to be emerging is that racemization is generally similar among proteins with minor exceptions. If racemization could be correlated with a specific property such as secondary structure (α-helical, β-pleated sheet, etc.) or polar amino acyl residue content, for instance, a useful tool for predicting processing damage would exist.

Effect of pH on Racemization. When racemization over the range of pH 3.3 to pH 12.8 was studied, it was observed that most amino acid residues in the proteins tested racemized to similar extents at a given pH (Figure 2). However, interesting differences were observed between the relatively easily racemized serine and Asp–Asn residues. In the case of LPC and ISP, serine began racemizing above basal levels even at pH 7.0 and was signficantly racemized at pH 8.5, a region of relatively moderate alkalinity. However, the other protein, LA, showed no serine racemization in this range. These differences in seryl racemization could be a result of differential denaturation of the proteins in this pH range; i.e., while LPC and ISP are begining to denature, LA may remain relatively intact. At pH 10, while differences persist, all of the proteins show a great deal of seryl racemization (25–37%), and at pH 12.8, racemization of serine is essentially complete in each protein, probably because complete denaturation occurs under such strongly alkaline conditions.

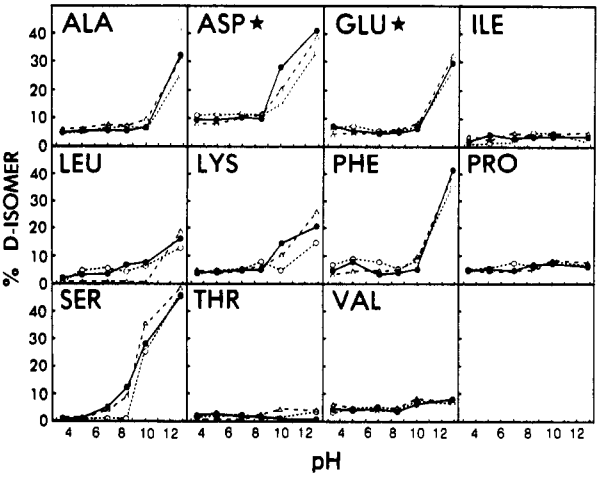

Figure 2. Effect of pH on racemization of LPC (●), LA (○), and ISP (△). Samples were held at 85 °C for 2 h (★ indicates combined value for the acid and amide).

Asp–Asn racemization, on the other hand, did not exceed basal levels in any of the proteins unitl pH 10 was reached. Again, LA showed the least racemization. An intermediate level of Asp–Asn racemization was observed for ISP and the greatest inversion was suffered by LPC. Lower racemization of Asp–Asn compared to serine was observed for each of the proteins at pH 10 and 12.8, and differences in Asp–Asn racemization similar to those observed at pH 10 persisted at pH 12.8 rather than converging to a maximum value as did serine. In a study by Masters and Friedman (1979), of four proteins subjected to alkali treatment (casein, lactalbumin, soy protein, and wheat gluten), LA also showed the least racemization of Asp–Asn. This could be due to differences in inherent, structure-related susceptibility to racemization of the proteins that may involve denaturation (NB, in about half of the residues measured, LA was less racemized than ISP and LPC at pH 12.8).

Besides denaturation, solubility also may have an effect on racemization. For instance, zein has been observed to racemize significantly when exposed to 0.1 N $Ca(OH)_2$ (Schwass et al., 1983), while racemization of protein in corn used for tortilla preparation (similar alkali treatment) has been reported to be quite low (Tovar and Carpenter, 1982). While the zein is not highly soluble, the protein in the milled corn is protected from an extensive chemical reaction by the matrix of the milled particle. Of the proteins used in this study, ISP apparently is least soluble and LA is most soluble at alkaline pH. It is not clear whether reduced solubility has been caused by denaturation, however, and denaturation would be expected to permit easier racemization of a protein. Therefore, these racemization

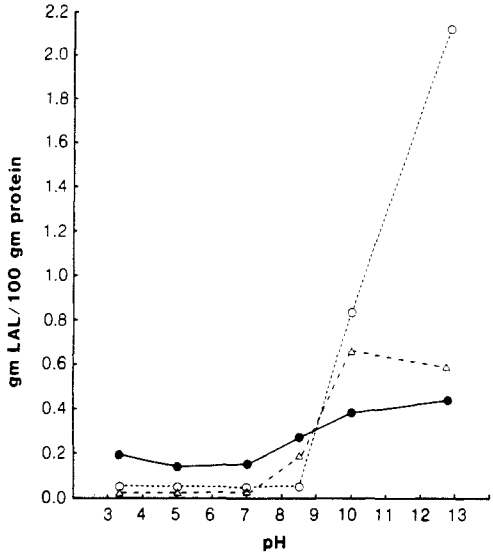

Figure 3. Effect of pH on LAL formation in LPC (●), LA (○), and ISP (△). Samples were held at 85 °C for 2 h.

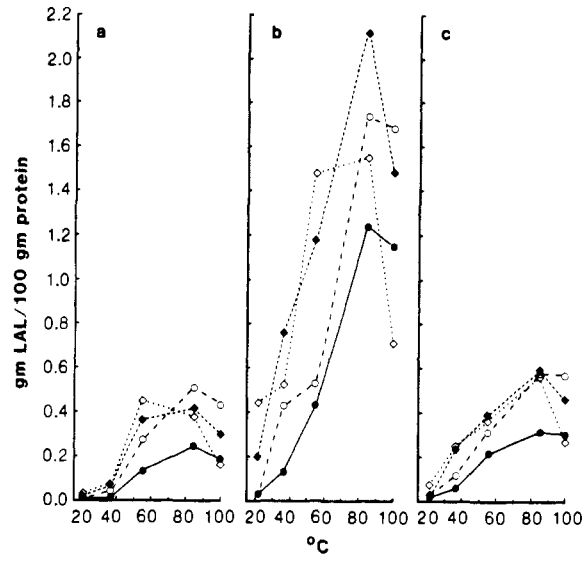

Figure 4. Effect of temperature on LAL content in proteins held in 0.1 N sodium hydroxide for 30 (●), 60 (○), 120 (♦), or 240 min (◇). Panel, a, alfalfa LPC; panel b, LA; panel c, ISP.

results may indicate that LA was least denatured of the proteins, a conclusion that is in accord with visible evidence of the protein solutions or suspensions. Differences in Asp–Asn racemization could also be explained if the asparagine:aspartate ratios (Asn:Asp) of the proteins followed the relationship $Asn:Asp_{LPC} > Asn:Asp_{ISP} > Asn:Asp_{LA}$. It has been observed that amino acids with amide R groups racemize faster under alkaline conditions than the corresponding carboxyl amino acids (Masters and Friedman, 1979), presumably because of the greater electron-withdrawing ability of the amide group (Charton, 1964). The values for asparagine content of these proteins are not available, as amides are hydrolyzed during the acid hydrolysis step. An analogous amide–carboxylate effect of similar magnitude was not observed for glutamine racemization. The difference in apparent inductive strength as it relates to alkali-induced racemization about the α-carbon between the amide and the carboxylate appears to be much diminished for the case of the longer homologue glutamate (Friedman and Masters, 1982).

In general, for each of the proteins and all of the measured residues except serine and Asp–Asn, little or no significant racemization above basal levels occurred below pH 10. Friedman and Masters (1982) observed similar results for casein when they measured glutamate (plus glutamine), phenylalanine, and aspartate (plus asparagine): only Asp–Asn showed appreciable racemization below pH 10.

Effect of pH on Lysinoalanine Formation. The influence of pH on the formation of LAL was investigated in the same group of samples described in the racemization studies. By further dilution of the derivatized samples to 500 μL with ethyl acetate, LAL could be determined on the OV-17-coated column. Significant formation of LAL was not observed in LPC, LA, or ISP at or below pH 8.5 (Figure 3). For the LPC and ISP, most LAL formation was observed when the pH of the sample was increased from 8.5 to 10.0. Little or no increase was noted in LAL formation over that occurring at pH 10.0 when these proteins were held at pH 12.8. Lactalbumin exhibited a similar increase in LAL content for samples held at pH 8.5 and 10.0, but formation was further elevated at pH 12.8. The continued increase in LAL content of LA at pH 12.8 also indicates that this protein may not have been as denatured at alkaline pH as the others in this study (see above). Studies by Haraguchi et al. (1980) indicate that LAL formation is dependent primarily upon intramolecular reactions, and complete denaturation at high pH would be expected to decrease LAL formation. Creamer and Matheson (1977) observed for casein a nearly exponential increase in LAL formation that rose from negligible at pH 10 to a maximum at pH 12 with a subsequent decrease of approximately 25% when the pH was raised further to 12.5. It is not clear whether Creamer's and Matheson's observation was unique to casein or is generally applicable. Our LAL determinations were made at relatively wide pH intervals that did not reveal decreases in LAL above pH 12. Also, we observed significant LAL formation between pH 8.5 and pH 10.0 in each of the proteins compared with little formation below pH 11.0 in casein (Creamer and Matheson, 1977). This may be due to the lack of cystine in casein: cystine degradation leading to LAL formation can occur at a pH of 10 or less whereas β-elimination reactions of serine occur above this pH (Karayiannis et al., 1979).

Effect of Temperature and Time on Lysinoalanine Formation. Lysinoalanine formation as a function of time and temperature in LPC, LA, and ISP is shown in Figure 4. Lysinoalanine determinations were performed on samples used for the racemization studies described above. The general trend was for parallel LAL formation in the three proteins, with LA exhibiting higher levels. However, rather than the steadily increasing amounts of LAL appearing with time that were observed in the samples held at 55 °C or less, samples held at 100 °C exhibit decreasing amounts of LAL with longer incubations. This phenomenon of decreasing LAL at high temperatures has been observed by several other laboratories (Provansal et al., 1975; Holstein, 1979; Hasegawa et al., 1981); however, temperature maxima have varied among laboratories. Of the temperatures tested in this study, the 85 °C treatment was most effective for producing LAL in these three proteins. Hayashi and Kameda (1980a) observed a temperature maximum of 60 °C for LAL formation in alkali-treated ribonuclease A. Hasegawa et al. (1981) observed that more LAL is produced during alkaline treatment of casein at 50 °C than at 95 °C (5 h), but this effect is due to breakdown of LAL during continued incubation at 95 °C. Because of the apparent differences among proteins in terms of temperature effects on LAL formation, detailed studies on LAL formation should be carried out on food

proteins over the range of 65–100 °C.

deGroot and Slump (1969) have suggested that LAL formation may be a useful index of protein damage. However, our results show that even when LAL is not observed, significant racemization may have occurred. Bunjampamai et al. (1982) have reported that the decrease in in vitro digestibility of casein observed after alkaline treatment is due primarily to racemization effects rather than to cross-linking by LAL. Our laboratory has reported that alkali-induced racemization of the lysine-free protein zein (therefore, LAL free) can significantly impair the uptake of partial hydrolysates of this protein by the gut (Schwass et al., 1983). Also, Jenkins et al. (1984) have reported that zein racemized during alkali treatment is substantially less available to the rat than the untreated protein. Therefore, racemization appears to play a substantial role in reduced bioavailability of alkali-treated proteins.

The results of this study have shown that serine and aspartate (plus asparagine) are the amino acid residues most susceptible to racemization in the proteins tested. For many proteins, serine racemization may be the most sensitive indicator of alkali-induced racemization damage because this residue is most labile at the lowest pH (8–10). While extremes of temperature or alkalinity tend to promote generally similar levels of racemization among proteins, differences in racemization are seen between proteins under less extreme conditions.

While each protein appears to exhibit a characteristic level of LAL formation under certain conditions (cf. LA and ISP in this study), racemization occurs in a distinctly different manner. With increasing time of alkaline treatment, racemization increases as well, but LAL formation appears to decrease if the treatment occurs at sufficiently high temperatures. This effect can be due to increased LAL breakdown under sufficiently harsh conditions (Creamer and Matheson, 1977). However, specific spatial relationships between lysine and dehydroalanine moieties appear to be important for LAL formation, espcially before denaturation, while the protein maintains some tertiary structure (Hasegawa et al., 1981). On the other hand, racemization is probably enhanced when the protein is entirely denatured. This interpretation is supported by our results with LA that show that it is most resistant to racemization but LAL formation is highest. In this way, the differences in denaturation between proteins may be responsible for differences in racemization that are observed at intermediate alkalinities (cf. serine, Figure 2).

Therefore, it is important that we understand how specific protein materials react under various alkaline conditions. Because alkali-induced reactions can affect nutritional quality, alkali-treated food proteins should be assayed for racemization and cross-linking on a routine basis. This may allow decreases in nutritional quality due to alkaline or high-temperature processing conditions to be minimized.

Abbreviations Used: Asp–Asn, combined value for asparate and asparagine: Asp:Asn, ratio of aspartate to asparagine; Glu–Gln, combined value for glutamate and glutamine; HCl, hydrochloric acid; ISP, isolated soy protein; LA, lactalbumin; LAL, lysinoalanine; LPC, leaf protein concentrate.

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Registry No. LAL, 18810-04-3; L-Ser, 56-45-1; L-Ala, 56-41-7; L-Asp, 56-84-8; L-Glu, 56-86-0; L-Ile, 73-32-5; L-Leu, 61-90-5; L-Lys, 56-87-1; L-Phe, 63-91-2; L-Pro, 147-85-3; L-Thr, 72-19-5; L-Val, 72-18-4; sodium hydroxide, 1310-73-2.

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Desorption and Adsorption Isotherms of Meat–Salt Mixtures

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Water binding by muscle proteins in the presence of different levels of salt was studied. Biceps femoris muscle was ground and dialyzed against distilled water. Increasing levels of salt were added to seven aliquots. Different levels of salt were added to each of seven aliquots of ground muscle. Half of each aliquot was taken for desorption studies; the other half was freeze-dried for adsorption studies. Isotherm data were obtained by equilibration to saturated standard salt solutions. Samples containing no salt had similar adsorption and desorption equilibrium moisture values and salt alone showed no sorption below $0.75a_w$ and a large hysteresis effect above $0.75a_w$. Increasing the salt content dramatically increased the moisture content at comparable a_w above 0.75, and the salt–muscle protein mixtures showed hysteresis.

Water and protein interactions have been intensively investigated over the last 10 years (Karel, 1973; Kuntz and Kauzmann, 1974). Early studies on the sorption of water vapor by proteins (Sponsler et al., 1940; Shaw, 1944; Bull, 1944; Pauling, 1945) found that the amount of water adsorbed depended on the number and availability of the two types of hydrophilic groups binding water molecules through hydrogen bonding. Cassie (1945) and Pauling (1945) showed that water vapor was adsorbed by these hydrophilic groups at lower water activities (a_w) and that multimolecular water adsorption occurs at higher a_w.

It has been found that the amount of water adsorbed by proteins at an a_w of 0.5 or below fits the Langmuir adsorption isotherm (Bull, 1944) and that the multimolecular adsorption of water at these a_w levels can be predicted by the BET equation (Brunauer et al., 1938; Shaw, 1944; Pauling, 1945; Guggenheim, 1966; Rochester and Westerman, 1976). However, Iglesias and Chirife (1976) and Labuza (1975) found that the BET equation does not accurately estimate the moisture conent at high a_w values (greater than 0.5) such as those associated with the physical and chemical deterioration of foods. Analysis of the system becomes more complicated with the addition of other components such as salt.

One of the many effects of sodium chloride in food systems is that it decreases a_w (Ingram and Kitchell, 1967). Most processed meat products contain sodium chloride, which adds sensory, functional, and preservation properties to the products. The trend toward development of more intermediate moisture meat products has placed a greater emphasis on understanding the equilibrium moisture characteristics of the meat proteins in conjunction with NaCl. The physical and chemical properties of many proteins are affected by ionic strength (Arakawa and Timasheff, 1982); thus, salt content affects the water binding properties of the proteins, which in turn affect food preservation. Previous studies have found that the water binding properties of proteins such as lysozyme (Hnojewyj and Reyerson, 1961), soy protein (McCune, 1981) and casein (Hardy and Steinberg, 1984) vary with the sample preparation and whether the equilibration was done from the wet or dry state.

The objective of this study was to obtain sorption isotherms for meat proteins containing different levels of salt in order to increase our knowledge of the effect of salt on meat systems under both desorption and adsorption conditions.

MATERIALS AND METHODS

Preparation of Samples. Bovine biceps femoris muscle was removed 8 h after slaughter, and excess fat and connective tissue were removed (Figure 1). The muscle was ground through a 3 mm hole plate and blended for 10 s in a Waring blender with 2 volumes of cold water. The homogenate was exhaustively dialyzed against three changes of cold water for a total of 3 days to remove inorganic salts and small molecular weight compounds. The dialysate contained 2 mM sodium azide to inhibit bacterial growth. The dialyzed sample was then split; one aliquot was freeze-dried and served as the dry control (sample 8, Table I), and the other aliquot was concentrated under vacuum at 4 °C over $CaCl_2$ in order to remove excess water and thus shorten equilibration time. An aliquot of this concentrate was removed for the wet control (sample 0, Table I). The concentrate was then divided into seven parts, and salt was added at different levels (samples 1–7, Table I). After salt addition, the samples were stored at 20 °C for 6 h; half of each sample was utilized for the desorption study, and the other half was freeze-dried for the adsorption study.

Isotherms. The isotherm data were obtained by using the proximity equilibration cell described by Lang et al. (1981) modified as specified below. All measurements were obtained at 5 ± 1 °C, and the different saturated salt solutions and all a_w values, some of which were taken from the literature and some of which were measured in this study, are given in Table II. All desorption and adsorption

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