Effect of γ-Irradiation on Lysinoalanine in Various Feedstuffs and Model Systems

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Changes in lysinoalanine (LAL) content in various animal feedstuffs and model systems by irradiation were investigated. LAL was detected in unirradiated animal feedstuffs such as fish meals, fish solubles, and bone meal. The amounts varied 0.3~0.6, 1.0, and 2.9 μmol/100 mg of sample, respectively. But LAL was not detected in soybean meal or feather meal. LAL contents in these animal feedstuffs remained unchanged by irradiation levels up to 5 Mrd (50 kGy). LAL in dilute solution (35 μmol/100 mL of 0.01 M phosphate buffer, pH 7.4) was decomposed exponentially by irradiation. The G value for decomposition was 2.84. But LAL was stable under irradiation in the dry state. In protein solutions, LAL was decomposed by irradiation as in the buffer solution. Under similar irradiation conditions losses in lysine, histidine, and arginine did occur. There was no formation of LAL by irradiation, regardless of the protein source (bovine serum albumin, ovalbumin, lysozyme), concentration (0.2 ~ 10%), or pH (7 ~ 13). These results show that irradiation is useful for proteinaceous foods and animal feed preservation especially from the standpoint of the LAL formation problem.

Lysinoalanine (LAL) [N^{ϵ}-(DL-2-amino-2-carboxyl)-L-lysine] is an unusual amino acid found in alkaline-treated proteins. LAL cross-linking results from the interaction of lysine residue with the dehydroalanine residue produced during decomposition of phosphoryl- or glycosylserine and cystine or cysteine (Bohak, 1964; Whiting, 1971; Asquith and Carthew, 1972). It is reported that LAL is formed by alkaline and heat treatment of purified proteins such as lysozyme and ribonuclease (Bohak, 1964; Hayashi and Kameda, 1980), soybean protein (Finley and Kohler, 1979; Saio and Murase, 1975), casein (Friedman et al., 1981; Hasegawa and Okamoto, 1980), and others (Fujimaki et al., 1980; Murase and Goto, 1977; Shetty and Kinsella, 1980). LAL is nephrotoxic to the rat (Woodard and Short, 1973; De Groot and Slump, 1969), but it is not yet known whether it is toxic to farm animals or man. At a minimum, however, LAL can cause a nutritional problem since it represents a corresponding decrease in available lysine and a loss in digestibility (De Groot and Slump, 1969; Friedman et al., 1981).

We have been investigating the effectiveness of irradiation of animal feeds and feedstuffs for the prevention of microbiological problems and the extension of shelf life (Kume et al., 1981, 1983a,b; Ito et al., 1981, 1983). For this method of sterilization to be acceptable, however, it is necessary to test for changes in composition following irradiation to ensure the wholesomeness of irradiated feeds and feedstuffs. Irradiation can cause the formation of unusual amino acids (Garrison, 1981). Cross-linking of proteins is known to result from irradiation (Yamamoto, 1977). However, there is little information about the effect of irradiation on LAL.

This paper describes changes in LAL content following irradiation of various animal feedstuffs. For this purpose several model systems have been employed.

MATERIALS AND METHODS

Materials. Commercial feedstuffs including fish meals, fish solubles (concentrated liquid products from fish waste), bone meal, soybean meal, and feather meal were obtained from the local market. Ovalbumin (grade V) and bovine serum albumin (crystallized and lyophilized) were purchased from Sigma Chemical Co., and lysozyme (6 times crystallized) was purchased from Seikagaku Kogyo Co., Ltd. LAL was obtained from Miles Laboratories Inc.

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Table I. LAL Content in Various Feedstuffs and the Irradiation Effect

	moisture content, %	LAL content, μmol/100 mg of sample: unirradiated	LAL content, μmol/100 mg of sample: 5.0 Mrd
bone meal	7.3	2.9	3.0
fish meal A	8.0	0.6	0.6
fish meal B	8.0	0.3	0.4
soybean meal	13.8	ND[a]	ND
feather meal	8.1	ND	ND

[a] Not detected.

Irradiation. The feedstuffs were irradiated at room temperature under air-equilibrium conditions with a 76 kCi (2.8 pBq) cobalt-60 slab source. Authentic LAL and protein solutions were irradiated at 0 °C under air-equilibrium conditions. The dose rates used were 0.1~1.0 Mrd/h (1~10 kGy/h) as determined by Fricke dosimetry.

Amino Acid Analysis. The samples for amino acid analysis were hydrolyzed in 6 N HCl in evacuated, sealed tubes at 110 °C for 24 h. Amino acid analysis was performed on a Shimadzu LC-3A high-performance liquid chromatograph equipped with an ISC-07/S1504 column. The amino acids were separated and eluted by using 0.2 N sodium citrate buffer containing 5% of ethanol (pH 3.21), 0.2 N sodium citrate buffer (pH 4.25), and 0.9 N sodium citrate buffer (pH 5.20) at a flow rate of 0.5 mL/min and a column temperature of 55 °C. The elution times of individual amino acids were established by using mixtures of pure amino acids. The o-phthalaldehyde (OPA) reaction at 60 °C was used for the detection of amino acids by a fluorescence detector FLD-1 fitted with a 360-nm excitation filter and a 450-nm cutoff emission filter (Ishida et al., 1981).

RESULTS AND DISCUSSION

LAL Content in Various Feedstuffs and Its Irradiation Effect. LAL can be formed by heat treatment without alkaline treatment (Sternberg and Kim, 1975). Since various feedstuffs are usually heat-treated during preparation, it was anticipated that the feedstuffs could contain LAL. As shown in Table I, LAL was present in commercial fish meals and bone meal while none was detected in soybean meal or feather meal. These amounts of LAL were not changed by 5 Mrd of irradiation. Table II shows the change in basic amino acid content including LAL in fish meal and fish solubles by irradiation. The contents of LAL and of other amino acids of the fish

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Table II. Irradiation Effect on LAL and Basic Amino Acid Content in Fish Meal and Fish Solubles

	moisture content, %	dose, Mrd	amino acid content, μmol/100 mg of sample			
			LAL	Lys	His	Arg
fish meal C	8.5	unirradiated	0.3	17.6	11.3	22.5
		1.0	0.4	17.8	11.4	21.8
		5.0	0.4	18.8	12.0	22.4
fish solubles	46.4	unirradiated	1.0	8.0	4.2	7.8
		1.0	0.9	7.8	4.1	7.3
		5.0	1.0	8.2	4.2	7.3

Table III. Changes in Amino Acid Content in Bovine Serum Albumin (BSA) and Ovalbumin (OVA) by Irradiation

protein	concentration, %	dose, Mrd	amino acid content, residues/mol of protein			
			LAL	Lys	His	Arg
BSA		unirradiated	ND[a]	59.4	16.8	24.6
	0.2	1.0	ND	50.5	14.1	21.2
		5.0	ND	33.5	11.9	14.7
OVA		unirradiated	ND	17.8	7.4	12.9
	0.2	1.0	ND	16.3	5.4	11.6
		5.0	ND	11.1	5.2	7.9
	1.0	1.0	ND	17.0	6.6	12.7
		5.0	ND	13.4	6.6	9.1
	10	1.0	ND	17.6	7.6	13.1
		5.0	ND	17.4	6.9	13.0

[a] Not detected.

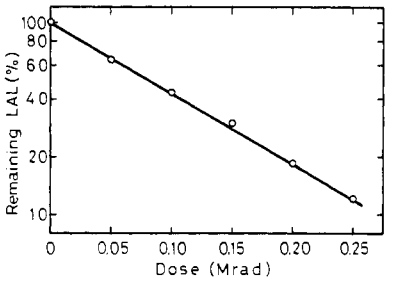

Figure 1. Decomposition of LAL irradiated in dilute aqueous solution. LAL solution (35 μmol/100 mL of 0.01 M phosphate buffer, pH 7.4) was irradiated at 0 °C under an air-equilibrium condition.

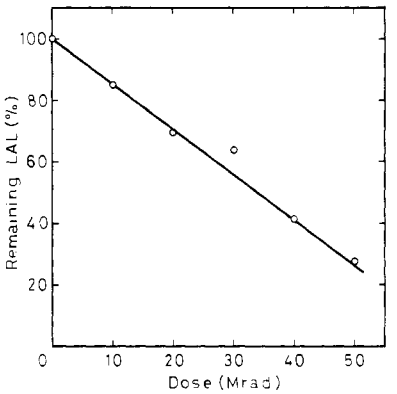

Figure 2. Decomposition of LAL irradiated in the dry state. Lyophilized LAL was irradiated at room temperature under an air-equilibrium condition.

solubles and fish meal are different, but no change in the amino acids was observed in either sample with irradiation up to 5 Mrd. These results indicate that some feedstuffs such as fish meals, fish solubles, and bone meal contain LAL (0.3~2.9 μmol/100 mg of sample), while no accumulation or decomposition of LAL resulted from irradiation in any of the examined feedstuffs.

Decomposition of Authentic LAL by Irradiation. Free amino acids are known to be more radiosensitive than bound amino acids in proteins or feedstuffs (Diehl and Scherz, 1975). Therefore, the effect of irradiation on an authentic LAL sample was studied. Figure 1 shows the change in LAL content when irradiated in a dilute solution (35 μmol/100 mL of 0.01 M phosphate buffer, pH 7.4). LAL decreased exponentially and the G value (number of molecules changed for each 100 eV of energy absorbed) for decomposition was calculated as 2.84 from a D_{37} dose. In general, the G values of amino acid decomposition are in the range of 1~10 (Diehl and Scherz, 1975). LAL is not as sensitive to radiation as sulfur-containing amino acids such as cysteine (G = 9.3). Figure 2 shows the decomposition of LAL irradiated in the dry state. LAL decreased linearly but 27% remained even at 50 Mrd. These results indicate that LAL is decomposed easily in dilute solution but is stable in the dry state.

Change in LAL Content in Protein Solutions by Irradiation. It is known that irradiation can cause proteins to be cross-linked and their amino acid compositions changed. Therefore, it was thought important to determine whether or not LAL was formed in protein solutions by irradiation. Table III shows the changes in basic amino acid composition when bovine serum albumin and ovalbumin were irradiated in 0.01 M phosphate buffer, pH 7.4. The contents of lysine, histidine, and arginine were decreased by irradiation up to 5 Mrd in both proteins at 0.2% concentration while LAL was not detected. The contents of amino acid of ovalbumin by irradiation were decreased to a less extent when the protein concentration was increased from 0.2% to 10%. It has been reported that protein concentration generally has no influence on the formation of LAL by alkaline treatment (Hayashi and Kameda, 1980). But a high yield of LAL has been observed at high protein concentration during preparation of "dope", which is an alkaline solution of soybean protein for production of spun fiber (Katsuta et al., 1982). The protein concentration could affect the formation of LAL if formation is due to intermolecular cross-linking as reported by Hasegawa et al. (1981). However, irradiation caused no formation of LAL even at concentrations of protein up to 10%.

The effect of pH on irradiation was examined with lysozyme. Table IV shows the changes in basic amino acid composition of lysozyme irradiated in a 0.3% solution at pH 8.0 and 13.0. The changes in lysine and arginine content were the same at both pHs, but there was no

Table IV. Irradiation Effect on Basic Amino Acid Content of Lysozyme

pH	dose, Mrd	amino acid content, residues/mol of protein			
		LAL	Lys	His	Arg
	unirradiated	ND[a]	5.7	1.0	10.6
8.0	0.1	ND	6.0	1.1	10.8
	1.0	ND	5.4	0.8	9.8
	5.0	ND	4.4	0.3	8.7
13.0	0.1	ND	5.6	1.2	10.6
	1.0	ND	5.3	1.1	9.9
	5.0	ND	4.2	1.0	6.5

[a] Not detected.

Table V. Irradiation Effect on LAL and Basic Amino Acid Content in Heat-Denatured Lysozyme[a] at pH 13

dose, Mrd	amino acid content, residues/mol of protein			
	LAL	Lys	His	Arg
unirradiated	2.8	3.1	1.2	10.9
0.1	2.7	2.9	1.0	11.3
1.0	2.1	2.1	1.1	9.4
5.0	1.5	1.5	0.9	5.5

[a] Lysozyme was heated at 40 °C for 4 h before irradiation.

significant change in histidine at pH 13.0. As shown in this experiment, the radiosensitivity of amino acids is pH dependent. No formation of LAL was observed at either pH. Table V shows the changes in LAL content caused by irradiation after LAL was formed as a result of heat treatment at 40 °C for 4 h in 0.2 N NaOH solution. The LAL content decreased with increasing irradiation dose, but the change in LAL content was almost the same as for the other basic amino acids. From these results, it can be concluded that LAL is not formed by irradiation of protein solutions, regardless of the protein source (bovine serum albumin, ovalbumin, or lysozyme), concentration (0.2~10%), or pH (7~13). On the other hand, LAL formed by heat with alkaline treatment of the proteins was removed by irradiation. However, it is impossible to decompose LAL selectively without affecting other basic amino acids since the radiosensitivity of LAL is similar to that of other amino acids.

CONCLUSION

LAL as a model system was decomposed in dilute aqueous solution and in protein solution by irradiation, and its sensitivity to irradiation was found to be similar to that of other amino acids. LAL is protected in feedstuffs from irradiation because feedstuffs are a complex matrix of many components with high concentration and have a low moisture content. On the other hand, LAL was not formed in protein solutions and feedstuffs by irradiation. Therefore, irradiation is a more appropriate method for sterilization of feedstuffs than heating. This is especially true from the standpoint of the LAL formation problem.

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Registry No. LAL, 18810-04-3; lysine, 56-87-1; histidine, 71-00-1; arginine, 74-79-3; lysozyme, 9001-63-2.

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