Studies with Radioactive Tracers. XVI. The Fate of Glycine-2-^{14}C during Breadmaking[1]

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ABSTRACT

Bread was made with glycine-2-^{14}C incorporated into the baking formula. The radioactivity was measured in the various fractions obtained in the breadmaking process and in the basic, acidic, and neutral fractions of the 80% ethanolic extracts of the crust and crumb. The basic fractions of these extracts were examined by paper chromatography. The results are compared with those obtained in a previous study in which glycine-1-^{14}C was used. It is concluded that decarboxylation of the glycine, such as by the Strecker degradation, is an important process, but much of the resulting C-2 fragment remained fixed as nonvolatile compounds in the finished bread. Some radioactive components of higher mobility than glycine observed in the paper chromatograms were found to be compounds containing the C-2 carbon and not C-1 of glycine. These results substantiate the occurrence of Maillard-type browning reactions in breadmaking and suggest the probability that the Strecker degradation of glycine, at least in part, did not occur immediately, but rather took place after the formation of reaction products between reducing sugars and the amino acid.

In an earlier study on the fate of glycine-1-^{14}C during breadmaking (1), evidence was obtained in support of the occurrence of Maillard-type browning reactions, the initial stages of which involve condensation reactions between reducing sugars and amino acids (2,3,4,5). The presence of radioactive reducing sugars in the finished bread when bread was made with sucrose-^{14}C (6,7) or with starch-^{14}C (8) incorporated in the baking formula further confirmed the availability of reducing sugars to take part in Maillard reactions. In the present work with glycine-2-^{14}C, studies analogous to those made with glycine-1-^{14}C (1) were carried out to provide further information, especially with regard to the fate of the C-2 moiety of the glycine molecule during the making of bread.

MATERIALS AND METHODS

Except for replacing glycine-1-^{14}C with glycine-2-^{14}C, the procedures used in

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[1]Contribution from the Department of Chemistry and Chemical Engineering, University of Saskatchewan, Saskatoon, Saskatchewan, Canada. For paper XV, see Lee, C.C., and Lai, T.-S., Cereal Chem. 47: 598 (1969).

the present work are similar to those employed in the previous study (1); some modifications in experimental details are noted below. The methods of fermentation, baking, and collecting of the various fractions during the breadmaking process have been described by Lee and Chen (6). In the previous work (1), the crust and crumb of the finished bread, after vacuum distillation (6), were extracted with water and the extracts fractionated into basic, acidic, and neutral fractions by passage through ion-exchange columns (6). In the present work, boiling 80% ethanol instead of pure water was used in the extraction. In the paper-chromatographic examination of the active basic fractions, descending development of the chromatograms was used instead of the ascending technique employed in the previous work (1). The basic fractions originating from the previous study with glycine-1-^{14}C were also investigated by descending chromatography to compare the results with those obtained from glycine-2-^{14}C.

RESULTS AND DISCUSSION

Activity Distributions

The distributions of ^{14}C-activity in the various fractions obtained are shown in Tables I and II.

Comparison of the data in Table I with the analogous results obtained from glycine-1-^{14}C (Table I, ref. 1) showed a pronounced difference—that essentially no active CO_2 was evolved when bread was made with glycine-2-^{14}C. This is to be expected, since in the decomposition of glycine via processes such as the Strecker degradation, CO_2 would arise only from the C-1 carbon. From the work with glycine-1-^{14}C (1), about 20 and 40% of the original glycine-1-^{14}C activity remained with the crust and crumb, respectively. In the present work with glycine-2-^{14}C, the corresponding residual activities in the crust and crumb were about 35 and 57%. These findings indicate that both the C-1 and C-2 carbons of glycine, and the C-2 carbon after decarboxylation, were present in the bread. However, the proportion of C-2 remaining was greater than that of C-1, suggesting a relatively greater extent of decarboxylation, with the resulting C-2 fragment fixed as some nonvolatile

TABLE I. ACTIVITY DISTRIBUTIONS IN BREADMAKING WITH GLYCINE-2-^{14}C

Fraction	Activity		Percent	
	Loaf I mμc	Loaf II mμc	Loaf I %	Loaf II %
Glycine-2-^{14}C	170,000	170,000	100.0	100.0
Fermentation condensate	trace	trace	...	...
Fermentation CO_2	trace	trace	...	...
Oven-vapor condensate	402	320	0.2	0.2
Oven-vapor CO_2	trace	trace	...	...
Crust distillate[a]	99	104	0.06	0.06
Crust residue[b]	60,400	56,980	35.5	33.5
Crumb distillate[a]	77	46	0.05	0.03
Crumb residue[b]	93,600	99,695	55.1	58.6
			90.9	92.4

[a]From vacuum distillation described earlier (see ref. 6).

[b]Residue after vacuum distillation.

TABLE II. ACTIVITY DISTRIBUTION IN 80% BOILING ETHANOL EXTRACTS OF VACUUM-DISTILLED CRUST AND CRUMB

				Fraction from Extract		
		Total	Extract	Basic	Acidic	Neutral
Crust[a]	Activity (mμc)	117,380	39,325	32,250	2,730	2,018
	Relative distribution (%)	100.0	33.5	27.5	2.3	1.7
	Percent based on original glycine-2-^{14}C	34.1	11.9	9.5	0.8	0.6
Crumb[b]	Activity (mμc)	193,295	108,256	92,742	2,468	1,198
	Relative distribution %	100.0	56.0	48.0	1.3	0.6
	Percent based on original glycine-2-^{14}C	56.7	31.8	27.2	0.6	0.3

[a]About 94% of the activity in the 80% ethanolic extract of the crust was recovered as basic, acidic, and neutral fractions after treatment with ion-exchange resins.

[b]About 89% of the activity in the 80% ethanolic extract of the crumb was recovered as basic, acidic, and neutral fractions after treatment with ion-exchange resins.

compounds in the finished bread. Theoretically, in a Strecker degradation of glycine, C-2 would appear as volatile formaldehyde (9,10,11,12). The present data would suggest that at least some of the glycine-2-^{14}C could have undergone reactions of a condensation type before the occurrence of decarboxylation, or the formaldehyde could have undergone condensation reactions immediately upon its formation and thus would not appear in the vapor phase. The occurrence of decarboxylation in the products formed between reducing sugars and alpha-amino acids would be in accord with the general picture of Maillard-type reactions (2,3,4,5), with Strecker degradations taking place during the later stages of browning reactions. The total recoveries of activity for loaf I and loaf II, respectively, were 90.9 and 92.4%, considerably greater than the recoveries of 70.0 and 66.4% noted in the work with glycine-1-^{14}C (1). This observation confirmed the earlier suggestion (1,6) that the loss in activity likely was due to untrapped volatile materials, largely active CO_2, in the study with glycine-1-^{14}C.

In the fractionation of the crust and crumb extracts into basic, acidic, and neutral fractions, boiling 80% ethanol instead of water was used as extraction solvent. Although water could extract more of the activity from the crust and crumb residues (compare Table II with Table II of ref. 1), the aqueous extract showed large losses after treatment with ion-exchange resins (1). Presumably water could extract more of the melanoidins, which were poorly recoverable from the ion-exchange resins. Use of 80% ethanol minimized this loss during the resin treatment to give the basic, acidic, and neutral fractions. It was noted that the effluents from the ion-exchange resin columns were more highly colored when aqueous extracts were fractionated, in accord with the suggestion that water extracted more melanoidins than 80% ethanol.

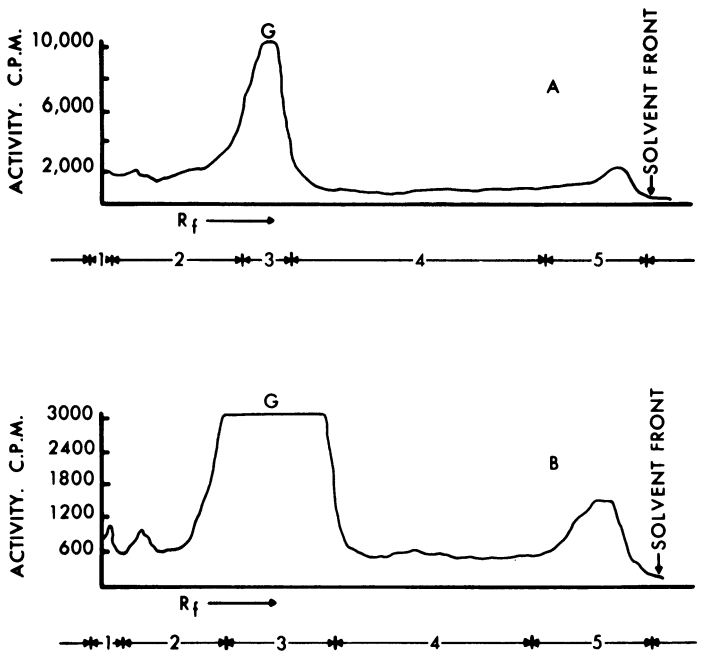

Fig. 1. Activity distributions on chromatograms of the basic fractions developed by the phenol-water solvent system. A, the basic fraction from the crust extract; B, the basic fraction from the crumb extract; G is glycine-2-^{14}C in this and subsequent figures.

Paper-Chromatographic Studies

The activity distributions on the paper chromatograms of the basic fractions of the crust and crumb from bread baked with glycine-2-^{14}C are shown in Fig. 1. The subdivision of each chromatogram into five subfractions, Ct-B-1 (crust, basic subfraction 1) to Ct-B-5 or Cb-B-1 (crumb, basic subfraction 1) to Cb-B-5, is also indicated on Fig. 1. These chromatograms were developed by the descending technique, with phenol-water (4:1) as solvent. For comparison, the basic fractions of the crust and crumb obtained from the work with glycine-1-^{14}C were also chromatographed in the same way. The most pronounced difference noted was that the highly mobile peak, observed near the solvent front in the work with glycine-2-^{14}C (Fig. 1, subfraction 5, $R_{glycine}$ = 3.00), was absent in the chromatograms of the basic fractions derived from bread baked with glycine-1-^{14}C.

Subfractions Ct-B-2, Ct-B-3, Ct-B-4, Cb-B-2, Cb-B-3, and Cb-B-4 were eluted separately and rechromatographed; the 1-butanol-acetic acid-water (4:1:1) solvent system was used. The activity distributions on the resulting chromatograms are shown in Figs. 2, 3, and 4. As noted in the studies with glycine-1-^{14}C (1), this technique of rechromatographing the various subfractions indicated the formation of numerous compounds arising from glycine during the making of bread. When the corresponding basic subfractions derived from the crust or crumb of bread baked with glycine-1-^{14}C were rechromatographed in the same way, by the descending paper-chromatographic technique, the most notable differences were found in the

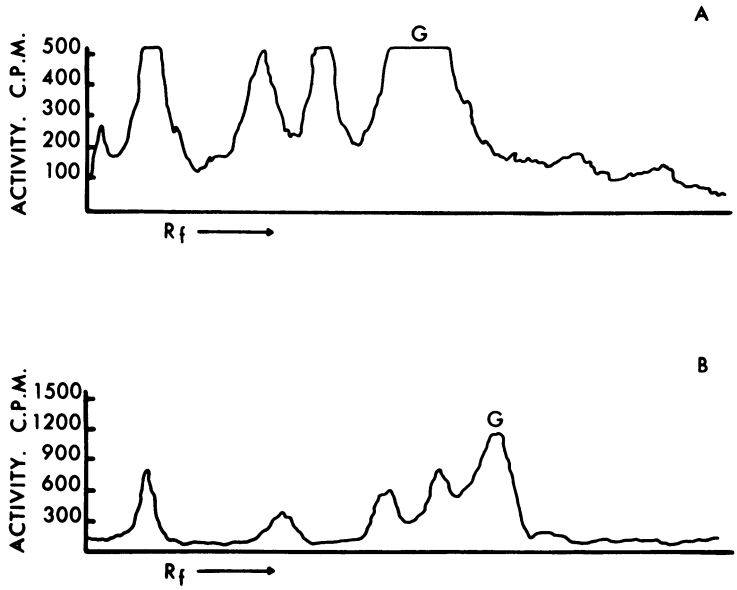

Fig. 2. Activity distributions on chromatograms of subfractions Ct-B-2 (A) and Cb-B-2 (B) developed by the 1-butanol-acetic acid-water solvent system.

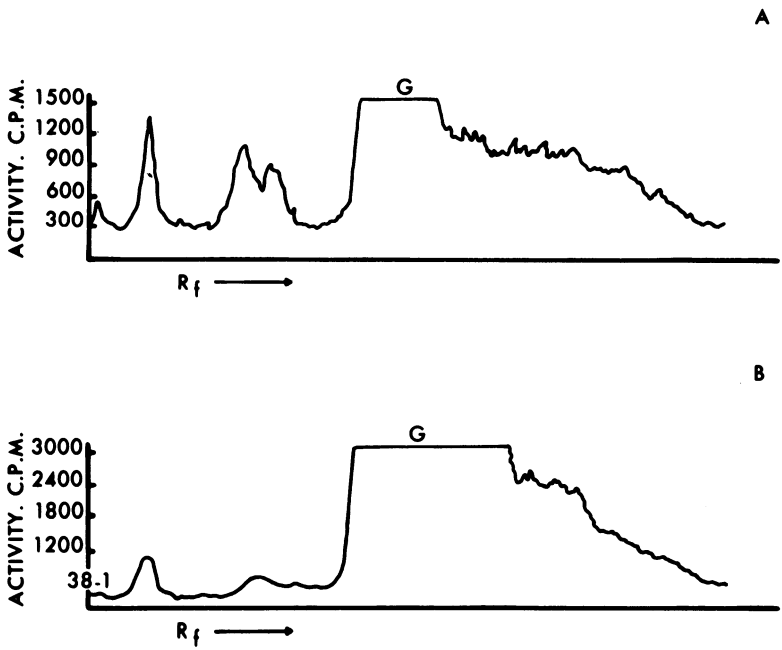

Fig. 3. Activity distributions on chromatograms of subfractions Ct-B-3 (A) and Cb-B-3 (B) developed by the 1-butanol-acetic acid-water solvent system.

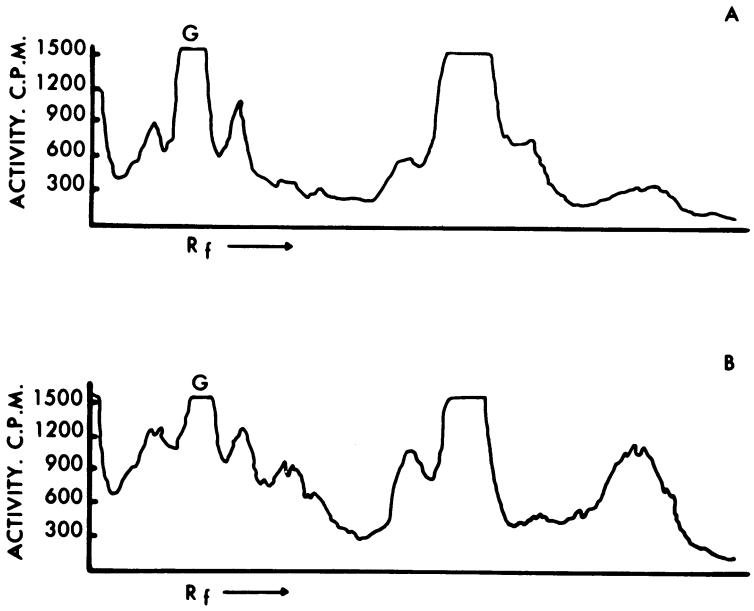

Fig. 4. Activity distributions on chromatograms of subfractions Ct-B-4 (A) and Cb-B-4 (B) developed by the 1-butanol-acetic acid-water solvent system.

materials with R_f values higher than that of glycine. Thus in the chromatogram of subfraction Ct-B-3 or Cb-B-3, the materials responsible for the highly active tailing following the large glycine peak, observed in the work with glycine-2-^{14}C (Fig. 3), were essentially absent when Ct-B-3 or Cb-B-3 was derived from glycine-1-^{14}C. Similarly, in subfraction Ct-B-4 or Cb-B-4, the chromatogram derived from the experiment with glycine-2-^{14}C showed a complicated activity distribution with many active peaks (Fig. 4); whereas, when the active ingredient was glycine-1-^{14}C, this subfraction showed only relatively low activity.

Some of the larger components shown in Figs. 2, 3, and 4 were subjected to acid hydrolysis and the hydrolysates rechromatographed (1). A general conclusion drawn from these hydrolytic studies is that those components with $R_{glycine}$ values of less than unity could give rise to glycine on hydrolysis, while those components with $R_{glycine}$ values higher than unity would not produce glycine on hydrolysis. These results, together with the observation that components with $R_{glycine}$ greater than unity did not show high activity when glycine-1-^{14}C was the source of radioactivity, indicated that the more mobile active compounds contained only C-2 of glycine, C-1 having been lost by decarboxylation processes. These findings demonstrated that C-2 of glycine was present in bread as compounds other than glycine or glycine condensation products. Such compounds could have been derived from the decarboxylation of the initial glycine condensation products or from the subsequent and rapid condensation of the formaldehyde resulting from the Strecker degradation.

Acknowledgment

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