

Studies with Radioactive Tracers. XIV. A Note on the Disulfide-Sulphydryl Interchange in Doughs Made with ^{35}S -Labeled Flour¹

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Recently Mauritzen (1) reported the results from a study on the incorporation of cysteine- ^{35}S , cystine- ^{35}S , and N-ethylmaleimide- ^{14}C into doughs, and the data were interpreted in terms of disulfide-sulphydryl interchange. On the other hand, Sullivan and Dahle (2) have stated that experiments with formamidine disulfide (FDS) have "cast doubt on the theory that the SH and SS groups of flour proteins interchange appreciably in the normal pH range of doughs." Sullivan², however, has informed one of us (C.C.L.) that studies in her laboratory with radioactive glutathione do show evidence of interchange during mixing, in agreement with the findings of Mauritzen (1). In the present Note, another tracer approach to the study of this problem is reported. This is based on the investigation of possible interchange reactions between inactive sulphydryl compounds and flour into which ^{35}S has been incorporated.

Aliquots of a sulfate- ^{35}S solution were injected into Thatcher wheat plants about 3 weeks before maturity, and the matured kernels were milled to give radioactive flour (3), which showed 19.0% protein ($\text{N} \times 5.7$) and 0.60% ash. The S-containing compounds in this flour, including those with SH and SS groups, as they exist naturally in the flour, would be labeled with ^{35}S . The amounts of ^{35}S as SH groups in the flour or dough were determined by treating the sample with N-ethylmaleimide (NEMI) followed by hydrolysis to ^{35}S -labeled S-succinyl-L-cysteine ($\text{I-}^{35}\text{S}$) (4,5,6).

The interchange reactions were carried out in a closed "dry box" under nitrogen. One-gram samples of the active flour and the appropriate aqueous solutions were placed in the box and flushed with nitrogen for 2 hr. Doughs were then prepared by hand-mixing with a spatula the 1.0 g. of flour with 1.0 ml. of water containing 0, 1.0, 2.0, 8.0, or 16.0 mg. of glutathione (GSH) or 30 mg. of thiolated gelatin (Gel-SH) (12 SH/100,000 g.). After 10 min. of mixing, a solution of 25 mg. of NEMI in 1.0 ml. of water was added and mixing was continued for 2 min. more. The resulting mixture was allowed to stand overnight. Each sample was made into a suspension: stirred for 15 min. in a total of 50 ml. of 6*N* HCl (allowance being made for the 2 ml. of water already present in the dough). Aliquots (1 ml.) of the suspension, each containing 2.0 mg. of NEMI-L-cysteine adduct as carrier (5,6), were evacuated and hydrolyzed as described previously (4,5,6) to give, among the hydrolysis products, $\text{I-}^{35}\text{S}$. The hydrolysates were subjected to paper-chromatographic separation with 1-butanol, acetic acid, water in the ratio of 4:1:1.8 (v./v.) as solvent. To obtain an effective separation

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²Sullivan, Betty, private communications.

of the cystine- ^{35}S and I- ^{35}S , three successive developments were employed, each chromatogram being developed in the solvent for 18 hr., air-dried, and then redeveloped for 18 hr. twice more. The activity distribution in each chromatogram was recorded and the ninhydrin-positive spots corresponding to cystine- ^{35}S and I- ^{35}S were cut out and counted to give the ratio of labeled S*S* to labeled S*H.

In addition to the experiments with the doughs described above, the S*H to S*S* ratio in the flour itself was ascertained by treating the flour directly with the NEMI solution without any GSH and without the 10 min. of mixing as a dough.

Two typical distributions of ^{35}S -activity in the chromatograms of the hydrolysates are shown in Fig. 1. Peaks corresponding to cystine- ^{35}S , I- ^{35}S ,

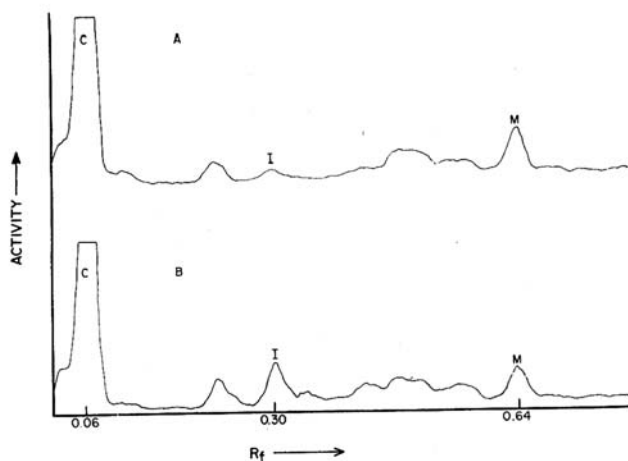


Fig. 1. Distribution of ^{35}S -activity in paper chromatograms of dough hydrolysates. A, dough mixed with no added sulfhydryls; B, dough mixed with 16.0 mg. GSH; C, cystine; I, S-succinyl-L-cysteine; M, methionine.

and methionine- ^{35}S were identified by comparison of each with the chromatographic behavior of authentic material. The unidentified peaks in the chromatograms indicate the presence of other ^{35}S -containing compounds in the hydrolysates, but the nature of these compounds was not investigated.

The activities of the spots corresponding to cystine- ^{35}S and I- ^{35}S for all of the experiments are summarized in Table I. Since I- ^{35}S resulted from the complete hydrolysis of the product of reaction between NEMI and the SH group of flour or dough proteins, the activity ratio of I- ^{35}S and cystine- ^{35}S is a measure of the relative amounts of S*H and S*S* in the system studied. It can be seen from Table I that the S*H to S*S* ratio of the flour is higher than that of the dough that was mixed without any added sulfhydryl. Apparently, under the conditions used in the present experiments, some loss of sulfhydryls has occurred during the 10 min. of mixing. When mixing was carried out in the presence of an inactive SH compound (GSH or Gel-SH), the ratio of active S*H to active S*S* ob-

TABLE I

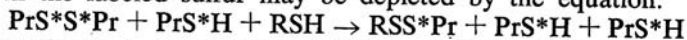
ACTIVITIES OF CYSTINE-³⁵S AND I-³⁵S IN HYDROLYSATES OF FLOUR OR DOUGHS THAT HAVE BEEN MIXED FOR 10 MINUTES IN THE PRESENCE OF INACTIVE SULFHYDRYLS

Compound		RSH ADDED		ACTIVITY ^a		ACTIVITY RATIO	
		mg.	μmole SH	I- ³⁵ S c.p.m.	Cystine- ³⁵ S c.p.m.	S*H	S*S*
Flour ^b	None			625	7,631	8.2	100
Dough	None			362	8,933	4.1	100
	None			443	10,600	4.2	100
	GSH	1.0	3.3	1,253	8,624	14.5	100
	GSH	2.0	6.5	1,296	7,283	17.8	100
	GSH	8.0	26	1,754	8,265	21.3	100
	GSH	8.0	26	1,558	8,017	19.4	100
	GSH	16.0	52	1,896	7,872	24.1	100
	GSH	16.0	52	1,826	8,158	22.4	100
	Gel-SH	30.0	3.6	527	7,983	6.6	100

^aEach value is the average from at least duplicate hydrolyses.

^bNo mixing as a dough.

served in every case was greater than that of the dough without added sulfhydryl. This observation is consistent with the occurrence of disulfide-sulfhydryl interchange reactions during the mixing of dough. The over-all changes in the labeled sulfur may be depicted by the equation:



While interchange between labeled S*H and S*S* of the dough proteins will not cause any net change in the activity ratio, the interchange between labeled protein disulfide (PrS*S*Pr) and the added inactive sulfhydryl compound (RSH) will cause some of the active S*S* to be liberated as S*H, while some inactive SH will become incorporated into disulfide as S*S. The net result will be an increase in the ratio of active sulfhydryl to active disulfide. The results (Table I) are entirely in accord with such a picture, especially in the experiments with added GSH. In the single experiment with 3.6 μmole of added Gel-SH, the rise in active S*H to S*S* ratio compared to that of the control dough was only about 50% (6.6:100 compared to about 4.2:100). On the other hand, 3.3 μmole of added GSH sharply increased this activity ratio to 14.5:100. These results suggest that molecular thiols, such as GSH, could easily come into reactive contact with the disulfide sites on a protein, whereas protein thiols, possibly because of their larger steric requirements, would approach and interchange with protein disulfides with much greater difficulty.

Acknowledgment

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Literature Cited

- MAURITZEN, C. M. The incorporation of cysteine-³⁵S, cystine-³⁵S, and N-ethylmaleimide-¹⁴C into doughs made from wheat flour. *Cereal Chem.* 44: 170-182 (1967).
- SULLIVAN, BETTY, and DAHLE, L. K. Disulfide-sulfhydryl interchange studies of wheat flour. I. The improving action of formamidine disulfide. *Cereal Chem.* 43: 373-383 (1966).

3. LEE, C. C., and REYNOLDS, L. M. Studies with radioactive tracers. VIII. The incorporation of S^{35} -labeled sulfate by maturing Thatcher wheat. *Cereal Chem.* 40: 487-492 (1963).
4. TKACHUK, R., and HLYNKA, I. Reactions of flour protein sulfhydryl with N-ethylmaleimide and iodate. *Cereal Chem.* 40: 704-716 (1963).
5. LEE, C. C., and LAI, T-S. ^{14}C -labeled N-ethylmaleimide as a reagent for the determination of flour sulfhydryls. *Can. J. Chem.* 45: 1015-1018 (1967).
6. LEE, C. C., and LAI, T-S. Studies with radioactive tracers. XI. The use of N-ethylmaleimide- $1-^{14}C$ in the determination of flour sulfhydryls and correlations between masked sulfhydryls and loaf volumes. *Cereal Chem.* 43: 620-630 (1967).

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In Diagram 1, p. 583, the word "RESIDUE" at upper left should be followed by the word "Discarded" (as in Diagram 2, p. 584, same relative position).



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