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The Incorporation of Nitrogen-15 into the Constituents of the Wheat Kernel[1]

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ABSTRACT

Ammonium chloride-^{15}N was administered to maturing wheat plants by injection into the top internode of the plants. Nitrogen-15 was readily incorporated into kernel components, as indicated by the observation of 4.67 atom % excess ^{15}N in kernel nitrogen. Gluten protein contained 5.00 atom % excess ^{15}N, whereas nitrogen of the salt-soluble kernel proteins contained 4.01 atom % excess ^{15}N. Examination of the nitrogen-15 distribution among eleven of the gluten amino acids showed difference in the labeling of individual amino acids. Glutamic acid (6.34 atom % excess ^{15}N) was most highly labeled, and proline, an amino acid derived from glutamic acid, contained 5.44 atom % excess ^{15}N. The other nine amino acids contained from 2.7 to 4.6 atom % excess ^{15}N. The processes involved in the incorporation of the tracer and the feasibility of utilizing nitrogen-15 for further study on the formation of cereal protein is discussed.

A number of experiments in this laboratory which have been based on the metabolism of carbon-14-containing compounds by maturing wheat plants have given information about pathways involved in amino acid formation (1) and about the sequence of formation of kernel proteins (2). A study of the metabolism of nitrogen-15-containing compounds would supplement these experiments by providing information about the pathways involved in nitrogen-15 metabolism. There are many references to the metabolism of nitrogen-15 compounds by higher plants, but the authors found few that deal specifically with wheat plants. However, the metabolism of ammonium phosphate-^{15}N by barley seedlings (3) has shown that after brief exposure to the tracer about 80% of the nitrogen-15 was in the alpha-amino groups of glutamine and asparagine. The amount of nitrogen-15 in the free ammonia was very low, indicating that this compound is very rapidly metabolized.

This report describes the results of experiments in which nitrogen-15 (as ammonium chloride) was injected into the stems of maturing wheat plants. The tracer is effectively incorporated into the kernel components, and, although it is widely distributed, there are significant differences between the labeling of individual amino acids.

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MATERIALS AND METHODS

Twelve wheat plants were injected with a solution of ammonium chloride-^{15}N, containing 96.9% ^{15}N (0.15 ml., 2.1 mg. per plant) just above the top internode when they were about 3 weeks from maturity (i.e., in the early dough stage). Another group (13 plants) was treated similarly with an aqueous solution of sodium carbonate-^{14}C (0.15 ml., 0.3 mg., 8.0 μc per plant) and a third group (12 plants) with sodium acetate-2-^{14}C (0.15 ml., 0.5 mg., 8.0 μc per plant). The solutions were administered with a small hypodermic syringe, and the resulting wound was sealed with collodion. The plants were harvested when mature and separated into kernels, chaff, upper stem, lower stem, and roots (4). The kernels were ground to a fine powder to pass a 40-mesh Wiley mill and separated into bran and endosperm particles. The endosperm was fractionated into starch, gluten, and salt-soluble protein.

A sample of the gluten (1.00 g.) was hydrolyzed with 5.7*M* hydrochloric acid for 20 hr. at 107°C. and fractionated into its constituent amino acids by the technique described by Hirs, Moore, and Stein (5). Another sample of gluten (0.5 g.) was refluxed for 2 hr. in 0.02*M* hydrochloric acid to hydrolyze the primary amide bonds of glutamine and asparagine (6). This hydrolysate was freeze-dried and then dissolved in 0.1*M* sodium hydroxide. The ammonia released was steam-distilled into 5 ml. of 0.07*N* sulfuric acid.

The total nitrogen in the gluten salt-soluble protein, bran, and the other plant parts was determined by a modified Kjeldahl procedure (7). Ammonia was distilled from the digests with a Markham distillation apparatus (8).

Nitrogen was prepared for mass spectrographic analysis by Kjeldahl digestion (7) of sufficient material to yield approximately 0.8 mg. of nitrogen. The digestion was carried out for 16 hr., and the ammonia was distilled into 5 ml. of 0.07*N* sulfuric acid. Quantitative oxidation of the ammonium sulfate to nitrogen by sodium hypobromite was carried out as described by Sprinson and Rittenberg (9). Nitrogen samples were assayed with an Associated Electrical Industries Ltd. mass spectrometer, type MS-3. The mass spectrometer was standardized each day with the nitrogen obtained by oxidizing ammonium chloride. The abundance of nitrogen-15 in each carrier amino acid was determined, and these values were used to calculate the atom percent excess nitrogen-15 in the enriched amino acids.

RESULTS AND DISCUSSION

Only part of the stem below the top node was harvested, but examination of this indicated that little net movement of nitrogen to the lower portions of the stem had occurred. The lower stem has a small nitrogen content, and its enrichment with nitrogen-15, although experimentally detectable, is also very small (Table I). The small amount of injected ammonia nitrogen in the lower stem (about 0.1% of total in parts examined) may even have resulted from accidental transfer of tracer to this area during the injection process. The nitrogen-15 content of the top internode was also low and, despite

TABLE I

DISTRIBUTION OF NITROGEN-15 INTO PLANT PARTS

PLANT PART	PART WT.	WT. OF NITROGEN	NITROGEN-15	NITROGEN-15	NITROGEN-15	NITROGEN-15
	mg./plant	*mg./plant*	*% excess*	*mg./plant*	*% of plant ^{15}N*	*mg./g. plant part*
Lower stem						
Water-soluble		0.037	0.15	0.06		
Water-insoluble		0.106	0.13	0.13		
Total	91.0	0.143	0.28	0.18	0.02	1.99
Upper stem						
Water-soluble		0.11	1.03	1.13		
Water-insoluble		0.26	1.04	2.70		
Total	195.0	0.37	2.07	3.83	0.50	19.64
Chaff						
Water-soluble		0.13	16.88	21.94	2.86	
Water-insoluble		0.75	3.95	29.63	3.87	
Total	142.0	0.88	20.83	51.57	6.73	363.0
Rachis						
Water-soluble		0.04	10.72	4.28	0.56	
Water-insoluble		0.12	4.77	5.72	0.74	
Total	36.0	0.16	15.49	10.00	1.30	277.7
Kernel	430.0	15.0	4.67	700.0	91.93	1,628.0

the fact that the isotope was injected here, it contained little more than 1% of the nitrogen-15 isolated from the mature plants.

Table I shows that the nitrogen-15 was extensively translocated into the ear of the plants. The soluble nitrogen of the chaff was particularly rich in nitrogen-15, an observation suggesting that although nitrogen is translocated into chaff it may not be readily utilized at this site. This observation

TABLE II

NITROGEN-15 AND CARBON-14 CONTENTS OF MAJOR KERNEL COMPONENTS

			INCORPORATION OF NITROGEN-15		INCORPORATION OF CARBON-14			
					Acetate-^{14}C		Na_2CO_3-^{14}C	
FRACTION	WEIGHT	NITROGEN	Atom % Excess	Percent of Kernel ^{15}N	Kernel Carbon-14	Sp. Act.	Kernel Carbon-14	Sp. Act.
	mg./plant	*mg./plant*		%	%	*μc/mg.*	%	*μc/mg.*
Kernels	430	15	4.67	100	100[a]	7.1	100[a]	1.7
Gluten	54	7.7	5.00	55	47	26.5	24	3.4
Salt-soluble protein	18	1.6	4.00	10	4.7	7.9	3.5	1.7
Starch	218	.....	.....	.....	11.4	1.6	34	1.2
Bran	71	1.3	4.41	7	8.7	3.7	9.1	1.2
Wash solutions					9.6		9.3	
Total				72	81.4		79.9	

[a] Kernels contained 3.02 μc/plant when labeled with acetate and 0.67 μc/plant when labeled with Na_2CO_3.

is analogous to those made in earlier experiments in which injection of carbon-14 compounds not utilized by wheat gave mature plants with an unusually large portion of the radioactivity deposited in chaff (3). Although soluble chaff nitrogen is highly enriched with nitrogen-15, this enrichment was achieved with the uptake of a relatively small amount of nitrogen-15 because of the low nitrogen content of chaff. There is no preferential movement of the nitrogen into chaff, and in fact its nitrogen-15 content per unit weight of mature chaff is only about one-third that of the protein-rich kernels (550 γ ^{15}N/g. for chaff compared with 1,700 γ ^{15}N/g. for the kernel). The rachis, too, contains nitrogen highly enriched with nitrogen-15. The recovery of 71% of the kernel nitrogen and 72% of the nitrogen-15 (Table II) indicates that the average enrichment of material in the discarded fractions (washings, etc.) was similar to that of the whole kernel. The kernels contain most of the plant nitrogen and about five-sixths of the nitrogen-15; Table II gives the nitrogen-15 content of the major kernel components as well as their carbon-14 contents. Nitrogen-15 enrichment ranges from 4 to 5 atom percent excess; the specific activities of the kernel constituents vary widely.

The compounds, ammonium chloride-^{15}N, sodium carbonate-^{14}C, and sodium acetate-^{14}C, were injected into separate groups of plants to compare, in parallel experiments, the distribution of the two tracers. The "specific" incorporation of acetate carbon into the protein fractions is similar to that observed in previous studies (2). This pattern is interpreted as a reflection of acetate utilization by way of the tricarboxylic acid cycle (2). The incorporation of carbonate is much less specific than acetate, but nevertheless it shows a greater variation than was observed for nitrogen.

The incorporation of nitrogen-15 into the amino acids isolated from an acid hydrolysate of gluten is given in Table III. The values are arranged in

TABLE III

NITROGEN-15 CONTENT OF AMINO ACIDS OF GLUTEN

AMINO ACID	ENRICHMENT	AMINO ACID	ENRICHMENT
	atom % excess		*atom % excess*
Glutamic acid[a]	6.34	Serine	4.48
Proline	5.46	Phenylalanine	3.94
Isoleucine	4.63	Tyrosine	3.85
Leucine	4.59	Aspartic acid	3.34
Alanine	4.59	Threonine	2.73
Glycine	4.41		

[a] Nitrogen of glutamic acid isolated from salt-soluble proteins contained 5.80 atom % excess nitrogen-15.

descending order, with glutamic acid the most highly enriched amino acid. This might well be expected in the present system if, as has been demonstrated in many systems (10), the amination of alpha-ketoglutaric acid is a major reaction for the conversion of ammonia to amino nitrogen. The alpha-amino group of glutamic acid is readily transferred to yield other amino acids by transamination of the appropriate keto acids. Since glutamic acid accounts

for at least 35% of gluten proteins, the initial appearance of nitrogen-15 in glutamic acid, coupled with the rapid incorporation of this amino acid into protein, offers a reasonable explanation for the higher labeling of glutamic acid.

Free ammonia present in the acid hydrolysate of gluten contains 9.2 atom percent excess nitrogen-15, a value considerably greater than any of the others from the kernel components. Most of the glutamic acid of gluten is present as glutamine, and since the acid hydrolysis of glutamine yields ammonia and glutamic acid, the results suggest that the amide nitrogen of glutamine is more highly enriched than the alpha-amino nitrogen.

The gluten hydrolysate (555 mg.) yielded 1.02 mmoles of glutamic acid, 0.02 mmole of aspartic acid, and 1.02 mmoles of ammonia. It is possible that some of this ammonia has been derived from nonprotein nitrogen, and thus there is a high value for the amide nitrogen tracer content. However, ammonia released by mild acid treatment of gluten from experiment 2 was enriched by only 6.2 atom percent excess. Although this result does not confirm the high enrichment observed in experiment 1, it is evident that the amide nitrogen is highly labeled.

Proline is closely related biosynthetically to glutamic acid, a fact that is reflected in carbon-14 tracer experiments by a similarity in labeling (1). Present experiments confirm this observation, since proline is second only to glutamic acid in enrichment with nitrogen-15. Arginine is also biosynthetically linked to glutamic acid and, like proline, is usually highly labeled with carbon-14 in experiments giving highly labeled glutamic acid. The enrichment of arginine nitrogen is not reported in Table III, because of experimental difficulties encountered in preparing a crystalline derivative. The measurements were made on a sample which was chromatographically pure. However, estimates of the enrichment of the alpha-amino nitrogen suggest a value near that for glutamic acid nitrogen. Furthermore, it was reliably estimated that the guanidino nitrogen of arginine had a higher enrichment than the alpha-amino nitrogen by a factor of 1.3. High enrichment of the guanidino group is not unlikely, because it is derived, in part, from carbamyl phosphate, whose nitrogen presumably comes from ammonia (10).

Leucine, isoleucine, and the short-chain amino acids all had an enrichment of about 4.5 atom percent excess nitrogen-15. Aspartic acid, which like glutamic acid actively participates in transamination reactions, was appreciably lower in nitrogen-15 content. This may indicate a relatively large or active aspartic acid "pool" with proportionally lower direct incorporation into gluten. Threonine has been shown to be derived from aspartic acid in wheat (11), and its labeling agrees with this observation.

The nitrogen-15 content of the salt-soluble protein (Table II) and of its component glutamic acid (5.80 atom % excess) was significantly lower than those of gluten and its glutamic acid counterpart. Thus, the total enrichment in nitrogen-15 of a particular protein depends on its amino acid composition as well as the nitrogen-15 enrichment of individual amino acids.

The present experiments have demonstrated that the techniques described introduce nitrogen-15 in measurable amounts into wheat kernel proteins. The

isotope is widely distributed among the amino acids, but there are significant enrichment differences between individual amino acids. The examination has been confined to plants labeled at a single time. However, the distribution among the separable proteins of plants given nitrogen-15 at different stages of growth might well vary in a manner similar to that observed in experiments with carbon-14. If so, data from such studies should provide insight into the sequence by which proteins are deposited in maturing kernels, as well as into the movement of nitrogen in various parts of the plant.

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