FORMATION OF STARCH IN WHEAT GRAIN[1]

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ABSTRACT

Wheat plants were either injected with randomly labeled sucrose-C^{14} or allowed to take up radioactive carbon dioxide in the light. In both groups of plants, the amylose became labeled much sooner than the amylopectin, clearly indicating that the path to amylose is more direct than the path to amylopectin. Although no evidence is available to suggest that amylose is not converted to amylopectin while these polysaccharides are being formed, the data suggest that amylose is not extensively converted to amylopectin once it has become incorporated into the granule.

In plants injected during daylight, radioactivity could be detected in the starch within 4 hours. Those injected during hours of darkness produced little or no labeled starch in 4 hours. Since no activity was found after 4 hours in the heads of plants injected at 11 p.m., translocation of carbohydrates to the head must have stopped during this period of the night.

Wheat plants which were grown under different day lengths had starch with the normal 25% amylose content. This indicates that the relative rate of formation of amylose to amylopectin remains the same whether the plant is in light or darkness.

Enzymatic conversion of amylose to amylopectin has been well demonstrated *in vitro* and excellent reviews are available (5,15). Recently two workers have doubted that amylose is converted to amylopectin during starch synthesis in the plant, and each has proposed a different mechanism for the simultaneous formation of amylose and amylopectin from a common precursor (7,15). Data obtained by examinations of the radioactivity of amylose and amylopectin from wheat starch 1 or 2 months after injection of the plant stems with glucose-1-C^{14} have been interpreted as evidence that amylopectin is formed from amylose (11).

The present paper reports the relative extent to which amylose and amylopectin incorporate carbon-14 when sucrose-C^{14} is injected into the wheat stem and when wheat plants are exposed to a $C^{14}O_2$ atmosphere in the light.

Amylose content of wheat starch produced under different day-lengths is reported.

Material and Methods

Wheat Plants Used. 1. Field: The wheat plants (Vermillion variety) injected with sucrose-C^{14} were grown in a filler plot on the Purdue Agronomy Farm.

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2. Greenhouse: Another variety of wheat, Monon, was planted November 18, 1959, in flats and was vernalized in a cold frame from November 24, 1958, until January 26, 1959. The plants were transplanted to 4-in. clay pots and grown in the greenhouse using supplemental light (10 p.m. to 2 a.m.). On March 26, 1959, eight plants were blooming and were moved to a room having lights controlled by a time clock. Half were grown to maturity with alternating periods of 2 hours light and 1 hour dark. The other four plants were subjected to alternating periods of 16 hours light and 8 hours dark. All were harvested at maturity on April 27, 1959; the starch was isolated, and the amylose content was determined. Thirty plants that bloomed between April 1 and April 7 were arranged into five samples in such a way that each sample had the same average blooming date and were given $C^{14}O_2$ on April 29, 1959.

3. Controlled climate: Wheat plants (Vermillion variety) were grown in the controlled-climate laboratory at 70°F. with 12-, 16-, and 20-hour day lengths. The starch was isolated and the amylose content was determined.

Administration of Carbon-14 to Wheat Plants. 1. Field: Randomly labeled sucrose-C^{14} (approximately 120 μc. per mg.) was prepared from a canna leaf surrounded by an atmosphere containing $C^{14}O_2$ according to the method of Putman, Hassid, Krotkov, and Barker (14). A 100-μl. aliquot of an aqueous solution of the sucrose-C^{14} was injected into the hollow part of each plant stem according to the method of Bilinski and McConnell (4). Plants were in the early dough stage about 16 days after flowering when injected with sucrose-C^{14}.

On June 26, 1958, approximately 17.5 μc. were given each plant. Those injected at 3 a.m. were harvested at 7 a.m.; those injected at 7 a.m. were harvested at 11 a.m.; and so on, at 4-hour intervals for a 24-hour period (all times are Central Standard Time). When harvested the heads were placed in plastic freezer bags and immediately frozen with dry ice.

On June 12, 1959, one series of injections started at 9 a.m. was completed by 10:45 a.m. The first eight samples received about 18.2 μc. per plant (ten plants per sample). The last five samples in this series received only 12.2 μc. per plant. Samples were harvested 0.25, 1, 2, 4, 9.6, 11.4, 13.3, 17.6, 56.2, 104, 147, and 336 hours after injection. The last sample was binder-ripe. Another series of four samples was injected from 6:30 p.m. to 7:05 p.m. on June 12. Each plant received only 7.9 μc. of sucrose-C^{14} of undetermined specific activity. The specific activity was probably not more than half that of the sucrose-C^{14} used in the other experiments. These samples were harvested 1, 3, 5,

and 9 hours after injection. The first sample was harvested at sunset.

2. Greenhouse: Thirty potted Monon wheat plants were placed in a 3 by 3 by 3-ft. frame covered with polyethylene plastic film 25 days after flowering (late dough stage). Two mc. of $BaC^{14}O_3$ were placed with 10 ml. water in a 125-ml. Erlenmeyer flask which was secured to the top of a magnetic stirrer. A thin-walled 1-ml. sealed tube of 85% lactic acid was placed in the flask with the $BaC^{14}O_3$. After the plastic cover was in place (11 a.m.) the magnetic stirrer was started from the outside, releasing the lactic acid into the $BaC^{14}O_3$. After 1 hour's stirring, the plastic was removed and the first sample (six plants) was taken. Another sample was taken at 3 p.m. and the other three samples were moved to a darkened hood. These last three samples were harvested at 4, 5, and 7 p.m.

Isolation and Fractionation of Starch. The grain was threshed by hand, while the heads were still frozen. The grains were crushed, a few at a time, in a glass mortar with a glass pestle. Small quantities of ice-cold 1% aqueous solution of sodium chloride were added as the grains were ground. The starch was washed through No. 25 bolting cloth. Vigorous shaking of the starch suspension with equal quantities of the sodium chloride solution and pentanol caused the protein to coagulate and separate at the interface. By repeated washing and sedimentation, the starch was separated from other impurities. The starch from the 1958 crop was fractionated immediately. The starch from the 1959 crop was stored in the freezer under 1% sodium chloride solution until fractionated.

The starch was fractionated without drying by a modification of the anaerobic leaching procedure of Baum, Gilbert, and Wood (2). In this fractionation, finely dispersed bubbles of nitrogen were passed for 30 minutes through 100 ml. of 1% aqueous sodium chloride solution containing about 0.5 g. of starch. Traces of oxygen were removed from the nitrogen by passing it through three traps of Fieser's solution (8) followed by a trap of concentrated sulfuric acid. Then the mixture was heated in a boiling water bath for 10 minutes followed by cooling to room temperature. The amylopectin formed a clear gel after 20 minutes' centrifugation at $2,400 \times g$. The amylopectin was washed five times with aqueous sodium chloride solution and redispersed as before. After five more washes with water, the amylopectin was suspended in about 15 ml. of water and poured into a blender containing 200 ml. of methanol. The precipitated amylopectin was washed with methanol and dried. Amylopectin thus prepared still contained 4–5% amylose as determined by electrometric iodine titration (1). The amylose solution was filtered through a medium sintered-glass

filter. Butanol in the critical amount (5% by weight) according to the method of Hiemstra, Bus, and Muetgeert (9) was added to the solution at room temperature. After overnight standing, the amylose butanol complex was removed as a tightly packed white gel by centrifugation at 2,400 × G. The supernatant solution was decanted and the amylose-butanol complex was dissolved in distilled water at room temperature. The second and third crystallizations with butanol occurred in 1 hour or less after the butanol was added. After three such crystallizations, the complex was dissolved in 30 ml. of water and slowly poured into a blender containing 250 ml. of methanol. The precipitated amylose was washed three times with methanol and dried. Amylose so prepared bound the same amount of iodine as a standard corn amylose prepared by the usual procedures. The starch of the 1959 crop was fractionated by the same procedure, except that a phosphate buffer (10) was substituted for the sodium chloride solution in the dispersions.

Determination of Radioactivity. Starch, amylose, and amylopectin were oxidized to carbon dioxide and plated as barium carbonate by a modification of the method of Claycomb, Hutchens, and Van Bruggen (6). An excess of sodium hydroxide was placed in the receiver and the titrations were omitted, since aliquots were not taken and dilutions were not made. The sample weight was chosen to obtain about 30 mg. per $cm.^2$ on an aluminum planchet 1.25 in. in diameter. Activity was measured in a thin-window gas flow Geiger tube (D 47 Nuclear, Chicago). Appropriate corrections for background and coincidence were applied.

Determination of Amylose Content of Starch. Grain from the wheat grown under different day-lengths was dry and was soaked 24 hours in water before grinding. The rest of the isolation was as previously described. Starch was washed with 85% methanol and extracted 24 hours with 85% methanol in a micro-Soxhlet apparatus. The starch was then dried and moisture was determined. Amylose was determined by a modification of the colorimetric method of McCready and Hassid (12). A starch sample containing 100 mg. of dry starch was weighed into a 250-ml. volumetric flask, 100 ml. of distilled water were added and the material was autoclaved 2 hours at 15 lb. per sq. in. Upon cooling, 25 ml. of 1*N* potassium hydroxide solution were added and the flask swept with nitrogen and stored, tightly stoppered, in the refrigerator. After room temperature was reached, water was added to bring the amount to 250 ml. Five milliliters were pipetted into a 200-ml. volumetric flask and 100 ml. of water added. Hydrochloric acid, 0.1*N*, was added to adjust pH to approximately 5 (about 5 ml.). Two

milliliters of iodine solution were added (0.2% I_2, 2.0% KI) and the solution was made to volume with water. The blank was made the same but without starch. The solution was read at 610 mμ in a spectrophotometer. Amylose content was determined by reference to a calibration chart previously prepared.

Results

Specific activities of wheat starch amylose and amylopectin from plants injected at 9 a.m. June 12, 1959, and harvested after various periods are shown in Table I. The activity of the starch and fractions rose very rapidly during the first 9 hours after injection. Smaller

TABLE I

SPECIFIC ACTIVITIES OF STARCH, AMYLOSE, AND AMYLOPECTIN FROM WHEAT INJECTED WITH SUCROSE-C^{14} 9 A.M. JUNE 12, 1959

(c/m $BaCO_3$ infinite thickness)

TIME AFTER INJECTION	STARCH	AMYLOSE	AMYLOPECTIN	RATIO OF SPECIFIC ACTIVITY, AMYLOSE/ AMYLOPECTIN
hours				
0.25	...	0	0	...
1.0	10	26	2	...
2.0	154	403	15	27.0
4.0	1,580	2,780	394	7.0
9.6[a]	7,770	12,700	2,370	5.4
11.4	9,150	16,000	3,830	4.2
13.3	9,500	13,900	5,330	2.6
17.6[b]	7,530	10,700	2,860	3.7
56.2	15,700	16,900	7,700	2.2
104.0	15,300	18,200	8,100	2.2
147.0	14,200	16,600	9,700	1.7
336.0	15,400	17,300	11,900	1.5

[a] Sample taken at sunset.
[b] Sample taken 30 minutes before sunrise.

changes in activity would not be apparent because equivalent amounts of radioactivity were not incorporated into each sample. The ratios of specific activity of amylose to that of amylopectin are also given in Table I. These values are free from variations due to different amounts of carbon-14 incorporated by each sample. The ratio of specific activity of amylose to amylopectin dropped very rapidly during the first 24 hours and then decreased only slightly during the next 2 weeks.

The specific activities of starch, amylose, and amylopectin from plants injected at 6:30 p.m. June 12, 1959, and harvested after definite periods are shown in Fig. 1. Here, too, the amylose is labeled much more quickly than the amylopectin.

Similar data are given in Fig. 2 for starch from plants exposed to

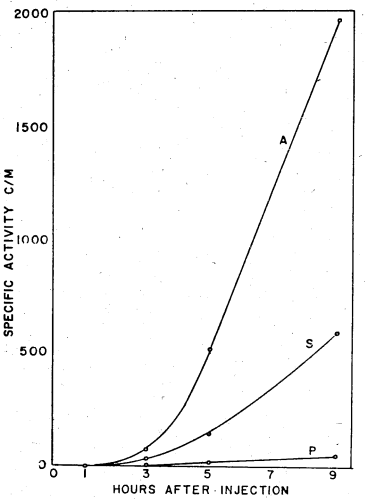

Fig. 1. Specific activity of (S) starch, (A) amylose, and (P) amylopectin from wheat injected with sucrose-C^{14} at 6:30 p.m. June 12, 1959.

an atmosphere containing $C^{14}O_2$. As previously observed, the amylose became labeled before the amylopectin.

Specific activities of wheat starch and its main fractions isolated 4 hours after plants were injected with sucrose-C^{14} at different times of the day are shown in Table II. Plants injected at 11 p.m. and harvested at 3 a.m. the following day had no measurable amount of radioactivity in the starch. In fact, no detectable amount of radioactivity

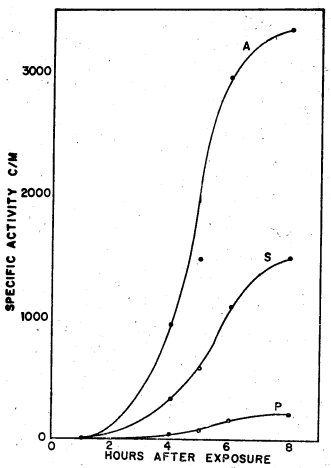

Fig. 2. Specific activity of (S) starch, (A) amylose, and (P) amylopectin from wheat exposed to $C^{14}O_2$ at 11 a.m. April 29, 1959.

TABLE II

SPECIFIC ACTIVITY OF STARCH, AMYLOSE, AND AMYLOPECTIN FROM WHEAT HARVESTED 4 HOURS AFTER INJECTION WITH SUCROSE-C^{14}
(c/m $BaCO_3$ infinite thickness)

TIME OF INJECTION	TIME OF HARVEST	SPECIFIC ACTIVITY		
		Starch	Amylose	Amylopectin
3 a.m.	7 a.m.	39	105	2
7 a.m.	11 a.m.	259	677	16
11 a.m.	3 p.m.	389	606	61
3 p.m.	7 p.m.	482	1040	110
7 p.m.	11 p.m.	10	43	0
11 p.m.	3 a.m.[a]	0	1	0

[a] June 27, 1958. All others June 26, 1958. Beginning of morning twilight 3:45 a.m., sunrise 4:18 a.m., sunset 7:23 p.m., end of evening twilight 7:56 p.m. Maximum temperature June 26, 72°F. Minimum temperature June 27, 54°F.

entered the heads during this night-time period. Specific activity of amylose was in all cases much greater than that of the amylopectin.

It is evident that the specific activity of the starch is greater than the activity of the fractions would indicate.

Amylose content of starch from Vermillion variety wheat grown in the controlled-climate laboratory under 12-, 16-, and 20-hour days was 25.5, 23.8, and 23.5%, respectively. Amylose content of Monon variety wheat starch, produced with lights on 2 hours, off 1 hour, and on 16 hours, off 8 hours, was 25.5 and 25.0%, respectively.

Discussion

Amylose becomes radioactive before amylopectin in wheat starch from plants given carbon-14. This is true regardless of the time of day that wheat plants in the early dough stage are injected with sucrose-C^{14}. This same sequence of events occurs when wheat plants in the late dough stage are exposed to $C^{14}O_2$. An unequivocal conclusion is that the path to amylose is more direct than the path to amylopectin. Conversion of amylose to amylopectin by the action of Q-enzyme, isolated from starch-forming tissues of plants, has been repeatedly demonstrated *in vitro*. This mechanism obviously provides a more direct path for amylose formation than for amylopectin formation. However, the data presented here do not eliminate the possibility of a different mechanism.

The gradual decrease in ratio of specific activity of amylose to amylopectin from 56 hours after injection to maturity is about the amount that can be expected from the increase in amylose content of the starch as the wheat approaches maturity (3). This suggests that amylose is not extensively converted to amylopectin, once it has become incorporated into the granule. No evidence is available to suggest that amylose is not converted to amylopectin while these polysac-

charides are being formed but before they are deposited in the granule.

The diurnal variation in rate of incorporation of carbon-14 into wheat starch following stem injections with sucrose-C^{14} can be explained by the variation in translocation rate. It is evident from the data that translocation of sucrose to the head drops to an immeasurably small rate between 11 p.m. and 3 a.m. This agrees with the observation (13) that translocation of sugars to the cotton boll is approximately four times greater during the day than during the night.

Amylose content is not affected by day-length. This seems to indicate that the rate of formation of amylose with respect to amylopectin is the same at night as during the day.

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Literature Cited

1. BATES, F. L., FRENCH, D., and RUNDLE, R. E. Amylose and amylopectin content of starches determined by their iodine complex formation. J. Am. Chem. Soc. **65:** 142–148 (1943).
2. BAUM, H., GILBERT, G. A., and WOOD, H. L. The fractionation of normal and mottled wheat starch by elution in the absence of oxygen. Viscometric properties of the amylose fraction. J. Chem. Soc. (London) **1955:** 4047–4051.
3. BICE, C. W., MACMASTERS, MAJEL M., and HILBERT, G. E. Wheat starch properties in relation to grain maturity. Cereal Chem. **22:** 463–476 (1945).
4. BILINSKI, E., and MCCONNELL, W. B. Studies on wheat plants using C^{14} compounds. III. The utilization of acetate for amino acid biosynthesis. Can. J. Biochem. Physiol. **35:** 357–363 (1957).
5. BOURNE, E. J. The biological synthesis of starch. Biochem. Soc. Symposia No. 11:3–16 (1953).
6. CLAYCOMB, C. K., HUTCHENS, TYRA T., and VAN BRUGGEN, J. T. Techniques in the use of C^{14} as a tracer. I. Apparatus and technique for wet combustion of nonvolatile samples. Nucleonics **7** (3): 38–41 (1950).
7. ERLANDER, S. A proposed mechanism for the synthesis of starch from glycogen. Enzymologia **19:** 273–283 (1958).
8. FIESER, L. F. Experiments in organic chemistry, 3rd ed., p. 299. D. C. Heath: Boston (1955).
9. HIEMSTRA, P., BUS, W. C., and MUETGEERT, J. M. Fraktionierung von Stärke. Die Stärke **8:** 235–241 (1956).
10. LANSKI, S., KOOI, M., and SCHOCH, T. J. Properties of the fractions and linear subfractions from various starches. J. Am. Chem. Soc. **71:** 4066–4075 (1949).
11. MCCONNELL, W. B., MITRA, A. K., and PERLIN, A. S. Studies on wheat plants using C^{14} compounds. VIII. Formation of amylose and amylopectin in the wheat kernel. Can. J. Biochem. Physiol. **36:** 985–991 (1958).
12. MCCREADY, R. M., and HASSID, W. Z. The separation and quantitative estimation of amylose and amylopectin in potato starch. J. Am. Chem. Soc. **65:** 1154–1161 (1943).
13. MASON, T. G., and MASKELL, E. J. Studies on the transport of carbohydrates in the cotton plant. I. A study of diurnal variation in the carbohydrates of leaf, bark, and wood, and the effects of ringing. Ann. Bot. **42:** 189–253 (1928).
14. PUTMAN, E. W., HASSID, W. Z., KROTKOV, G., and BARKER, H. A. Preparation of radioactive carbon-labeled sugars by photosynthesis. J. Biol. Chem. **173:** 785–795 (1948).
15. WHELAN, W. J. Starch and similar polysaccharides. *In* Encyclopedia of plant physiology, Vol. VI, pp. 154–240. W. Ruhland, ed. Springer-Verlag: Berlin (1958).