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MYO-INOSITOL SYNTHESIS IN GERMINATING SEEDLINGS

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Abstract—The conversion of ^{14}C D-glucose 6-phosphate to *myo*-inositol in wheat and bean seedlings has been examined. There was no incorporation of label in the early stages of germination, but ^{14}C was incorporated at later stages of development.

THE BIOSYNTHESIS of *myo*-inositol in microorganisms,[1] animals[2] and plants[3,4] proceeds from D-glucose 6-phosphate via cyclization to *myo*-inositol. Cell-free extracts, which synthesize *myo*-inositol from D-glucose 6-phosphate have been prepared from *Sinapis alba* and *Phaseolus vulgaris*, both 4 weeks old,[3] and from ripening rice grains.[7] In intact plants, the conversion of labelled D-glucose to *myo*-inositol has been observed in parsley[4] and 2-week-old *Sinapis alba*.[5,6] However, preliminary experiments with germinating wheat, 3 days after imbibition suggested that there was negligible conversion of D-glucose to free *myo*-inositol or lipid bound *myo*-inositol.[8]

In the present communication, these observations have been extended using both wheat and bean seedlings of various ages. Uniformly labelled D-glucose 6-phosphate was fed to beans at 3, 8, 15 and 22 days after imbibition and also to etiolated beans at 11 days and to wheat at 3, 7 and 28 days. Non-phytate *myo*-inositol was isolated and purified by paper chromatography prior to scintillation counting.

As shown in Table 1, there was substantial uptake of ^{14}C by beans at all stages and this uptake was lower in older plants. At 3 days, intact seedlings were used whereas at later stages plants with cut stems were fed. There was uptake by both methods. Three-day-old plants respired a higher proportion of absorbed ^{14}C (21% compared with 4–6%), but there was utilization in respiration at all stages. No label could be detected in the non-phytate *myo*-inositol of 3-day-old plants: the limit of detection was 0 04 mμc. The glucose 6-phosphate may have been partly or wholly hydrolysed to D-glucose before utilization. At later stages of growth in the light, label was detected in *myo*-inositol. Dark grown plants at 11 days showed no incorporation into *myo*-inositol. These also showed a higher proportion of conversion of absorbed ^{14}C glucose 6-phosphate into $^{14}CO_2$ (19%) than light grown plants of similar age. Intact wheat at 3 days (Table 2) showed a higher uptake of labelled

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TABLE 1 INCORPORATION OF ^{14}C FROM D-GLUCOSE ^{14}C(U)-6-PHOSPHATE INTO *myo*-INOSITOL IN BEAN SEEDLINGS

Age of plants (days)	Amount of D-G-^{14}C(U)-6-P applied (μc)	Amount of D-G-^{14}C(U)-6-P taken up in 24 hr (μc)	Amount of ^{14}C respired (μc)	Amount of ^{14}C converted to *myo*-inositol (mμc)	Non-phytate *myo*-inositol (mg/g dry wt)	Specific activity of *myo*-inositol (mμc/mg)
3	2 95	2 15	0 45	n s *	1 8	n s *
8	3 90	1 93	0 13	7 1	4 7	1 7
15	2 95	0 81	0 03	4 4	13 3	2 1
22	2 95	1 43	0 09	8 3	12 7	2 2
11 (etiolated)	3 00	1 41	0 26	n s *	2 3	n s.*

* Not significant.

glucose 6-phosphate than did older plants with cut stems and again showed a higher conversion to $^{14}CO_2$, but there was no incorporation of ^{14}C into non-phytate *myo*-inositol. In separate experiments with three day-old seedlings grown completely in the dark the same result was found. Wheat plants at 7 and 28 days did incorporate label into *myo*-inositol.

TABLE 2 INCORPORATION OF ^{14}C FROM D-GLUCOSE ^{14}C(U)-6-PHOSPHATE INTO *myo*-INOSITOL IN WHEAT SEEDLINGS

Age of plants (days)	Amount of D-G-^{14}C(U)-6-P applied (μc)	Amount of D-G-^{14}C(U)-6-P taken up (μc)	Amount of ^{14}C respired (μc)	Amount of ^{14}C converted to *myo*-inositol (mμc)	Non-phytate *myo*-inositol (mg/g dry wt)	Specific activity of *myo*-inositol (mμc/mg)
3	3 0	2 80	1 21	n s.*	0 45	n s *
7	3 0	2 55	0·45	0 2	0·58	0·4
28	2 95	1·19	0 07	0 8	0 84	1 9

* Not significant

These findings confirm the preliminary results previously obtained, that young wheat seedlings do not convert glucose to *myo*-inositol[8] and extend the observations to beans. Conversion was observed in older plants, in agreement with other data.[3–6]

The results suggest that wheat and bean seedlings, in the initial stages of germination, cannot convert D-glucose 6-phosphate to *myo*-inositol. If complete hydrolysis of ^{14}C D-glucose 6-phosphate to ^{14}C D-glucose by phosphatase took place then it would be D-glucose that cannot be converted to *myo*-inositol. Since some of the label appears as $^{14}CO_2$, either glucose 6-phosphate is not completely hydrolysed or hexokinase is operating. Therefore the block to the synthesis of *myo*-inositol is either the lack of D-glucose 6-phosphate cyclase (or *myo*-inositol phosphate phosphatase) or that it does not operate.

D-Glucose 6-phosphate serves as a substrate for four enzymes (Fig. 1), phosphohexoisomerase, phosphoglucomutase, phosphoglucodehydrogenase and phosphoglucocyclase.

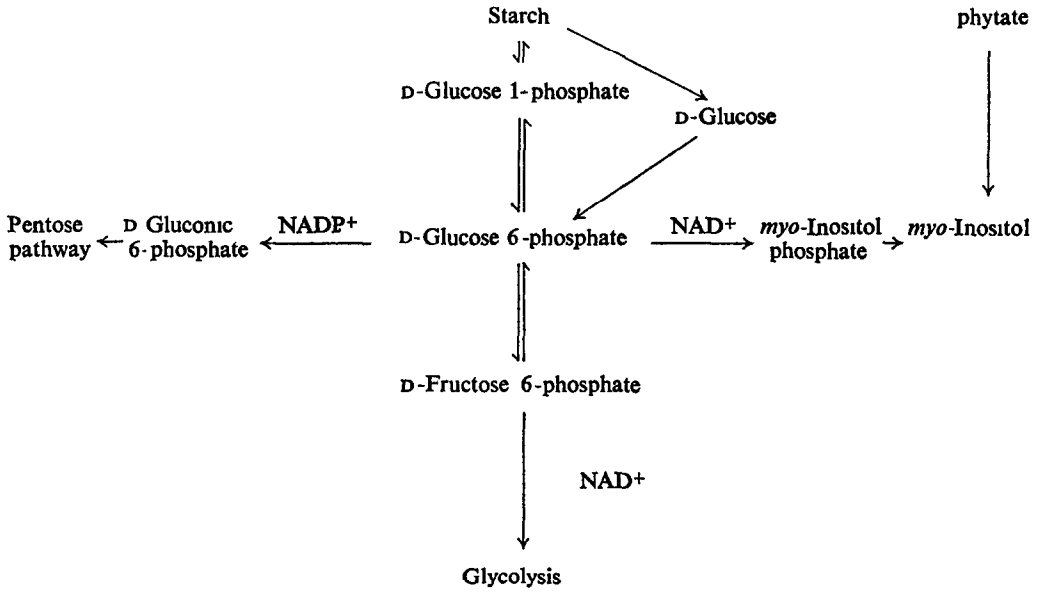

FIG 1. UTILIZATION OF D-GLUCOSE 6-PHOSPHATE AND THE ORIGIN OF *myo*-INOSITOL.

One of these, the cyclase, has a co-factor requirement for NAD^+ [3] for which it may compete with glycolysis. One interpretation of these results is that germinating seeds utilize stored phytate (Fig. 1) as a source of *myo*-inositol, instead of using the pathway from D-glucose 6-phosphate (or D-glucose), and this allows the maximum quantity of D-glucose 6-phosphate (or D-glucose) to be used for glycolysis in the early stages of high growth rate. The lack of conversion into *myo*-inositol in etiolated 11-day bean seedlings, when the proportion of glucose respired is also high, indicates that this behaviour is not limited to the period immediately after imbibition.

EXPERIMENTAL

Plant Material

Seeds of *Triticum vulgare* var. Mendos and *Phaseolus vulgaris* var Redlands Pioneer (dwarf) were steeped in 0·5% NaClO solution for 10 min, rinsed and washed in water for 1 hr They were grown in Perlite at 25° in a 13 hr photoperiod. After 7 days the plants were watered with Hoagland's solution. With 28-day-old wheat individual culms were used

Feeding of D-*Glucose-*^{14}*C(U)-6-phosphate*

Seedlings were incubated at 25° for 24 hr in a solution of uniformly labelled D-glucose-^{14}C-6-phosphate (0·5 μc/ml, 7·4 μc/mg) and unlabelled D-glucose 6-phosphate (0·5 mg/ml) Dry weight of seedlings was determined from a parallel experiment without added label. Beans at 3 days (with the split testas removed) and wheat at 3 days were fed with the roots and portion of the cotyledon immersed. All the other samples were cut at ground level and the stem immersed. Respired CO_2 was collected in NaOH solution, precipitated as $BaCO_3$ and counted

Extraction of Free myo-*Inositol and Lipid* myo-*Inositol from Seedlings*

The method was a modification of that previously described.[8] Seedlings were ground in 0·5 M $HClO_4$ in an all-glass Tenbroeck homogenizer (2–5 g seedling/40 ml acid) The homogenate was centrifuged

[9] E F L. J ANET and T. M. REYNOLDS, *Nature* **174**, 930 (1954).

(30,000 *g*, 30 min) and washed twice with 0 5 M $HClO_4$ (30 ml) and water (30 ml) The residual pellet was dried and extracted in a glass Tenbroeck homogenizer with $CHCl_3$–MeOH (1:1) (40 ml), centrifuged (30,000 *g*, 30 min) and washed with $CHCl_3$–MeOH (2 × 30 ml) The solvent was distilled under reduced pressure below 40° and the residue boiled for 40 hr in 6 M HCl The acid was removed by distillation under reduced pressure

The combined supernatants from the $HClO_4$ extraction were made 15 mM to EDTA and the pH adjusted to 7 with KOH After 18 hr at 4°, the precipitate was removed by centrifugation (30,000 *g*, 30 min) and the supernatant deionized (1RA-400 and AG50W-X4) The solution was made 0 75 M to H_2SO_4, boiled for 5 hr, neutralized with solid $BaCO_3$ and the pH raised to 11 0 with 25% NaOH. The suspension was centrifuged (2000 *g*, 30 min) and the residue washed twice (2 × 100 ml). The supernatants were reduced in volume by distillation under reduced pressure and the residue combined with the acid hydrolysate from $CHCl_3$–MeOH extraction. The volume was made up to 10 ml and oxidized for 2 hr in the dark with 0 05 M I_2 solution (30 ml) in a 0 1 M phosphate buffer (pH 11 3) (50 ml). The solution was deionized and reduced in volume to 2–3 ml. The remaining H_2O was removed by freeze-drying and the residue dissolved in 100 μl H_2O. After paper chromatographic purification (solvent acetone–H_2O, 17 3) activity was measured by liquid scintillation counting in 0·4% PPO, 0 005% POPOP in toluene Paper squares cut parallel to developed *myo*-inositol standards were used.

For estimation of *myo*-inositol content, a suitable aliquot was chromatographed with *myo*-inositol (1 μl–6 μl of 0·5 mg/ml). With these amounts spot area was proportional to concentration. The $AgNO_3$ spray of Anet and Reynolds[9] was modified to dip in both solutions Papers were fixed in 5% $Na_2S_2O_3$ solution.

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