Dormancy in Seeds of Charlock (Sinapis arvensis L.)

EARLY EFFECTS OF GIBBERELLIC ACID ON THE SYNTHESIS OF AMINO ACIDS AND PROTEINS

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ABSTRACT

Charlock (Sinapis arvensis L.) seeds were imbibed with 10 mM GA3 for 24 hours at 0°C. After equilibration at 25°C, a 5-fold increase in radioactivity in the amino acids labeled from 2-14C-acetate was observed within 2 hours. The total amount of amino acids was reduced to half, and the specific radioactivity increased approximately 10-fold, indicating a diversion of metabolites for amino acid and protein synthesis in GA3-treated seeds. The rate of incorporation of L-14C-leucine into protein was doubled. Autoradiographs showed that enhancement of protein synthesis was localized in the shoot and root meristems, the developing vascular tissues, and in the endosperm cells inside the testa.

The dormancy of charlock (Sinapis arvensis L.) seeds is imposed by the seed coats, which do not form a significant barrier to water uptake. The dormant embryo can be activated either by removal of the seed coat, which increases O2 supply and leaching of endogenous inhibitors (6, 7), or by treatment with a high concentration of GA3. The effect of GA3 is correlated with a very small increase in the respiration rate, therefore, growth is not limited by the low internal O2 concentration of the system (8, 9).

Tuan and Bonner (16) concluded that the genome of dormant cells was in a repressed state, and following this work, Jarvis et al. (10, 11) suggested the possibility that GA3 breaks seed dormancy by a mechanism of gene derepression. In dormant hazel (Corylus avellana L.) seeds treated with 10 μg ml-1 GA3, the first observed change was an increase in DNA template available for transcription and increased RNA synthesis in the embryonic axis after 11 hr. This preceded increase in RNA and protein synthesis and activity of enzymes involved in intermediary metabolism in the cotyledons (1, 14, 15). A delay of 12 to 14 hr was found in dormant Avena fatua L. grains imbibed with 10 μM GA3 before enhancement of RNA and protein synthesis (4, 5), which preceded germination (after 24 hr), and the synthesis of α-amylase in the aleurone layer (after 48 hr) (3).

The results of this work show early effects of GA3 on amino acid and protein synthesis during the breaking of dormancy of seeds of charlock (S. arvensis L.).

MATERIALS AND METHODS

Plant Material. Charlock seeds were surface-sterilized in 96% ethyl alcohol for 5 min and dried for 1 hr under UV light. Sterile seeds were imbibed in deionized water or 10 mM GA3 for 24 hr at 0°C, and afterward, washed thoroughly to remove the external source of GA3. Fifty seeds were incubated in 1 ml of deionized water containing 5 μCi 2-14C-acetate (3.2 Ci mol-1) plus a drop of 0.5 μg ml-1 each of penicillin, streptomycin, and mycelium, for 2 hr at 25°C. Fifty isolated embryos and seed coats were incubated separately in 1 ml of deionized water containing 1.25 μCi L-14C-leucine (278 Ci mol-1) plus a drop of bacterio-fungisstat, for 2 hr at 2°C. The seed parts were supplied with labeled leucine in the dark at 0.0118 atm O2; the O2 concentration at the surface of imbibed seeds was 0.0183 atm O2 (9).

Analytical Procedures. The seeds supplied with 2-14C-acetate were ground in anhydrous diethyl ether, and the ether-soluble substances separated by centrifugation at 1800g for 10 min. The residue was re-extracted with 80% ethyl alcohol for 1 hr at 65°C, and the ethanol-soluble substances separated similarly. The residue from the second extraction was resuspended in deionized water. Aliquots of each extract were dried on ground glass planchettes with and without 0.1 mM H2PO4 for 1 hr at 70°C, and 14C estimated with a Nuclear-Chicago gas flow detector. The ethanol-soluble substances were further fractionated by passing through a column (10 X 10 mm) of cation exchange resin (Dowex AG 50W-X8 hydrogen form) and of anion exchange resin (Dowex AG 1-X8 formate form) (2).

Incorporation into individual amino acids was estimated after separation by two-dimensional chromatography on a thin layer of silica gel H, using a mixture of butanol-glacial acetic acid-water, 4:1:1, v/v/v (solvent 1) and phenol-water, 75:25, w/w, with 20 mg NaCN added to 100 g (solvent 2) (13). Similar samples were separated on a column of Technicon chromobeads type C-2 eluted with standard citrate buffer, pH 2.875. 14C was estimated with a Beckman liquid scintillation system after adding Bray's scintillation fluid to amino acids adsorbed on silica gel H, and modified scintillation liquid (17) to column eluate containing amino acids. Measurements of the total amount of individual amino acids were made using a modified accelerated flow Technicon amino acid analyzer, with a limit of resolution of 5 nmol.

The embryos and seed coats supplied with L-14C-leucine were ground in ice-cold 0.2 M NaCl with 0.1 mM L-leucine, and the supernatant separated from the residue by centrifugation at 12,000g for 15 min. The residue was resuspended in deionized water. Aliquots were precipitated immediately with cold 15% trichloroacetic acid, or after incubation with and without pronase (1 mg ml-1, pH 8, for 30 min at 37°C). The precipitates were washed and dried on Millipore filters before estimation of 14C with a Nuclear-Chicago gas flow detector.

 Autoradiography. The distribution of 14C in seeds imbibed with water or GA3 and subsequently supplied with L-14C-leucine was examined by autoradiography (12). The seeds were fixed in 10% acrolein, passed successively into methyl cellosolve (ethylene glycol mono-methyl ether), absolute alcohol, 1-propanol

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Table I. Incorporation of 2-14C Acetate by Seeds
Incorporation was carried out for 2 hr at 25 C, following imbibition with water or 10mM GA3 for 24 hr at 0 C. A: seeds imbibed with water; B: seeds imbibed with 10mM GA3; C: enhancement. cpm per 50 seeds (150 mg dry weight) or seed parts.

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Table II. Distribution of Radioactivity from 2-14C Acetate among the Main Amino Acids in Seeds
Isotopic content was determined after 2 hr at 25 C, following imbibition with water or 10mM GA3 for 24 hr at 0 C.

Table III. Incorporation of 1-14C-leucine by Excised Embryos and Seed Coats
Isotopic content was determined during 2 hr at 25 C, following imbibition of intact seeds with water or 10mM GA3 for 24 hr at 0 C. cpm per 50 seeds (150 mg dry weight) or seed parts.

RESULTS
Incorporation of 2-14C Acetate. A consistently higher rate of incorporation of 2-14C-acetate was found in the seeds imbibed with GA3, which had several times the amount of 14C in both embryos and seed coats than the seeds imbibed with water (Table I). The amount of 14CO2 evolved from 2-14C-acetate was small compared with the 14C incorporated in the tissues of the imbibed seeds. No difference could be detected in the total CO2 estimated gravimetrically as BaCO3 (2.7 mg) as a result of imbibition with 10 mm GA3, but there was a significant increase of 2- to 3-fold in the specific radioactivity (evolved 14CO2 = 180 cpm seeds imbibed with water, 490 cpm seeds imbibed with GA3). Part of the 14C was found in ether-soluble substances, indicating some incorporation into lipid components. By far, the greater amount of 14C was found in amino acids, less in organic acids and sugar phosphates, and traces in neutral sugars. The incomplete recovery from the combined fractions of amino acids, organic acids, and sugars suggested that 14C was also incorporated into other unidentified components which were found mainly as positively charged materials (approximately 15% of recovered counts) associated with the amino acid fraction (Table II). Part of the 14C was incorporated into polypeptides and proteins in the residue, of which 93% in embryos and 70% in seed coats was water-soluble. The higher rate of incorporation of 2-14C-acetate into amino acids, polypeptides, and proteins indicates the diversion of metabolites for protein synthesis in seeds imbibed with GA3.

An analysis of the distribution of 14C in the amino acid fraction was made after separation by thin layer and column chromatography (Table II). Separation by TLC indicated that 2-14C-acetate was incorporated into all of the known amino acids, but most of them contained less than 3% of the total, and therefore, have not been listed individually in Table II. The distribution of 14C was similar with and without GA3. In the embryos, approximately 70% was in glutamic acid, and much smaller amounts in aspartic acid, glutamine, and alanine. The distribution in the seed coats was different from the embryos, as less was incorporated into glutamic acid (38%) and more into other amino acids. The higher rate of synthesis into glutamic acid in the embryos may have been connected with the subsequent renewal of cell expansion and cell division, which did not occur in the seed coats. The 5-fold increase in activity following imbibition with GA3 was associated with a reduction in the total amount of amino acids to approximately half, and an increase in the specific radioactivity by approximately one order of magnitude. The
embryo is comprised of about 455,000 cells (8), therefore, calculating as an average value for all of the cells, the total amino acid concentration was $8.56 \times 10^{-5}$ nmol/cell with GA$_3$ and $1.59 \times 10^{-4}$ nmol/cell with H$_2$O. The total amount of amino acids in the seed coats was much less than in the embryos, and the specific radioactivity was one or two orders of magnitude higher. However, calculating as an average value for all of the cells (about 18,000 living cells), the total amino acids were $6.24 \times 10^{-5}$ nmol/cell with GA$_3$ and $1.44 \times 10^{-4}$ nmol/cell with H$_2$O.

**Incorporation of L-$^{14}$C-Leucine.** Uptake of L-$^{14}$C-leucine by isolated seed parts at 25°C (embryos 11.68%, seed coats 8.29%) was much greater than by intact seeds (0.17%), since the resistance to penetration of amino acids through the outer part of the seed coat was avoided. L-$^{14}$C-Leucine was incorporated into salt-soluble and -insoluble polypeptides and proteins in the embryos which could be largely hydrolyzed with pronase (Table III). Much less was incorporated into salt-soluble proteins in the seed coats than the embryos, probably due to the smaller amount of amino acids present. Relatively more was in the insoluble material of the seed coats, which was not readily hydrolyzed by pronase. Although more than half of the $^{14}$C was in protein, the remainder appeared to be bound to constituents which strongly adsorbed amino acids. In seeds imbibed with GA$_3$, the incorporation of L-$^{14}$C-leucine into the soluble proteins of the embryos was doubled. The effect on incorporation into the soluble proteins of the seed coats, and of the insoluble proteins of all of the tissues was comparatively small.

Samples of this experimental material were examined by autoradiography. Figure 1 shows sections of an embryo cut obliquely in two parallel planes, the first through the cotyledons and shoot.

**Fig. 1.** Autoradiographs showing incorporation of L-$^{14}$C-leucine in the embryo and seed coat of a seed imbibed with 10 mM GA$_3$ for 24 hr at 0°C, and afterward exposed to labeled leucine for 2 hr at 25°C. Vertical section through the cotyledons and shoot apex (A) and cotyledons and root apex (B) ($\times$ 35), and seed coat (C) ($\times$ 450).
Dormancy in Sees of Charlock

apex (Fig. 1A), and the second through the cotyledons and root apex (Fig. 1B). The autoradiographs show that estimates of the total incorporation of L-14C-leucine into proteins in the embryo were a poor indication of incorporation in the tissues most affected by GA3. The cells of the shoot and root apex and developing vascular tissues constituted regions of much higher activity than the rest of the embryo. The higher activity in these cells could not have been due to an increase in supply of 14C from the source, because the cells at the surface of the meristems were not as active as those within (Fig. 2, A and B), and the high

![Autoradiographs showing incorporation of L-14C-leucine in the shoot apex (A) and root apex (B) of an embryo imbibed with 10 mM GA3 for 24 hr at 0 C, and afterward exposed to labeled leucine for 2 hr at 25 C (× 450). Shoot apex (C) and root apex (D) of an embryo imbibed with water (× 450).](image-url)
activity of the vascular tissues in the interior appeared to depend on the differentiation of the vascular elements. The surface cells generally had slightly more activity, and the cell walls also had more activity than the cell contents, indicating some difficulty in penetration into individual cells. The differences observed in the tissues of the embryo imbibed with GA$_3$ were much less apparent in the embryo imbibed with water (Fig. 2, C and D). The seed coat is comprised of the testa inside which is a single layer of living cells derived from the endosperm. Incorporation occurred only in this layer (Fig. 1C); the small scatter of silver grains on each side could have arisen through dispersion in the emulsion. All of the $14$C-leucine previously adsorbed in the outer part of the seed coat was removed by preparatory processes before autoradiography.

CONCLUSION

The results show enhancement of amino acid and protein synthesis in the cells of the meristems and developing vascular tissues of the embryonic axis and cotyledons, and in the endosperm cells inside the testa, with 10 mm GA$_3$. The results also show enhanced incorporation into lipid components. The effects were observed during 2 hr at 25 C after imbibition of dormant seeds with 10 mm GA$_3$ for 24 hr at 0 C. It is not at all clear what changes occurred during uptake of water and GA$_3$ at 0 C, but these may be assumed to be minimal, and the effects observed are early responses to GA$_3$ during the activation of dormant cells, and the breaking of dormancy of seeds of charlock.

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