Glucosinolate Uptake by Developing Rapeseed Embryos

Mark Gijzen, Ian McGregor*, and Ginette Séguin-Swartz

Agriculture Canada Research Station, Saskatoon, Saskatchewan, Canada S7N 0X2

ABSTRACT

Embryos excised from the seed of rapeseed (Brassica napus L.) accumulated glucosinolate from the culture medium. Uptake was saturable, subject to inhibition, varied with the developmental stage of the embryo but correlated with the time of accumulation of glucosinolates in situ. It is suggested that a carrier-mediated transport system is operating in the developing embryo.

During embryogenesis, glucosinolates accumulate in the seed of rapeseed (Brassica napus L.). At maturity, the embryo comprises from 80 to 90% of the seed dry weight and is the site where most of the glucosinolates contained in the seed are located (7, 14). The subcellular location of the glucosinolates within the embryo is not known for certain, although they are thought to be localized in globoid bodies associated with aleurone grains (17), structures which form within and eventually fill the vacuoles. Whether embryos have any synthetic capability, or simply act as a sink for glucosinolates synthesized in the pod or other plant parts, is not known.

Immature rapeseed embryos germinate when removed from the seed if placed in nutrient medium or water (3). Precocious germination may be prevented by increasing the osmotic potential of the medium or by adding ABA (4, 5). Immature embryos cultured on high osmoticum develop normally in that they continue to synthesize and accumulate embryo-specific storage protein (4). This investigation presents evidence that immature rapeseed embryos cultured on high osmoticum do not synthesize and/or accumulate glucosinolates if glucosinolates are absent from the culture medium, but can take up exogenously supplied glucosinolate in a carrier-mediated fashion. This ability to take up glucosinolate varies with development of the embryo, and may account for glucosinolate accumulation in situ.

MATERIALS AND METHODS

Plant Materials

Rapeseed (Brassica napus L. cv Golden) plants were grown in soil-free medium (15) in growth cabinets under an 18 h photoperiod with a photosynthetic photon flux density of 250 μE m⁻² s⁻¹ and a 22°C/18°C light/dark cycle. Illumination was provided by a combination of fluorescent and incandescent lights. Flowers were hand pollinated and the date of pollination was recorded.

Accumulation Experiments

Accumulation of glucosinolate in situ was examined from 11 to 40 DAP² by excising embryos from seeds at 2 to 4 d intervals. Accumulation of glucosinolates in vitro was examined by aseptically excising embryos from the seeds and cultivating 10 explants (6 × 1.5 cm) in 5 mL of solid Monnier’s culture medium (12) (filter sterilized, 0.4% agarose) according to the method of Crouch and Sussex (3) with and without the addition of 1.0 mM 2-propenyl-, 3-butenyl-, or benzylglucosinolate. Potassium salt of 2-propenylglucosinolate (sinigrin) was purchased from Sigma. The tetramethyl-ammonium salts of 3-butenylglucosinolate and benzylglucosinolate were purified from the seeds of rapeseed (Brassica campestris L. cv R500) and nasturtium (Tropaeolum majus L.), respectively, by the method described by Hanley et al. (6). Each plate constituted a sample.

Glucosinolate uptake by embryos over a 26 h period was examined by excising embryos from seeds, 10 to 20 per sample, and placing in 5 mL of medium containing 0.35 μM sucrose, 10 mM Mes/KOH buffer (pH 6.5), and 4.0 mM benzylglucosinolate. Benzylglucosinolate concentrations in the range of 0.1 to 15 mM were used to study the effect of glucosinolate concentration on uptake. The protonophores CCCP and 2,4-DNP, the respiratory chain inhibitor NaN₃, and the ATPase and Pase cofactor Mg²⁺ (in the form of MgSO₄) were added to the incubation medium to determine if these compounds have inhibitory or stimulatory effects on glucosinolate uptake.

To determine the relationship between glucosinolate uptake in vitro and glucosinolate accumulation by embryos during development in situ, uptake by embryos excised at various stages of development was compared to total glucosinolate content of embryos growing in situ. Uptake was assayed in embryos incubated in a medium containing 0.35 μM sucrose, 10 mM Mes/KOH buffer (pH 6.5), and 1.0 mM 2-propenylglucosinolate as substrate.

Uptake experiments were performed under fluorescent lights and at room temperature, 24°C and, with the exception of the variable time experiments, for 24 h. Uptake was expressed on a FW basis as μmol (g FW)⁻¹ h⁻¹. All inhibitors were obtained from Sigma.

Glucosinolate Determinations

Embryos were washed with 10 mL of distilled water, blotted dry on filter paper, weighed, and placed in test tubes containing 3.0 mL of hot distilled water heated in a boiling water

² Abbreviations: DAP, days after pollination; FW, fresh weight; CCCP, carbonyl cyanide m-chlorophenylhydrazone; 2,4-DNP, 2,4-dinitrophenol.
RESULTS AND DISCUSSION

Accumulation of glucosinolates in situ commenced at 16 DAP and rose rapidly for the next 24 DAP (Fig. 1). Embryos removed from the pod at 24 DAP and cultured in vitro failed to show a similar accumulation of glucosinolate. Addition of 2-propenylglucosinolate to the medium resulted in rapid uptake of this compound. After 3 d of culture, half of the 2-propenylglucosinolate originally in the medium could be accounted for in the embryos. The concentration of glucosinolate in these embryos was in excess of 20-fold that in the medium after 3 d of culture. Depletion of glucosinolate supply in the medium and large increases in FW of the embryos cultured longer than 3 d resulted in an effective decline in glucosinolate concentration in the embryos after 3 d of culture. Embryos also accumulated 3-butenyl- or benzylglucosinolate when cultured in the presence of these glucosinolates (Fig. 2). Of the glucosinolates tested, only 3-butenylglucosinolate is present in substantial amounts in situ; 2-propenylglucosinolate is present in trace amounts, and benzylglucosinolate is absent. That all these glucosinolates were accumulated by the embryos with similar efficiency indicates that the factor(s) responsible for their accumulation may have little specificity toward the side chain. Depletion of glucosinolate supply in the medium and large increases in FW of the embryos cultured longer than 3 d resulted in an effective decline in glucosinolate concentration in the embryos after 3 d of culture. This was substantiated by subculturing the embryos after 3 d in fresh medium with and without 2-propenylglucosinolate (Fig. 3). Without added glucosinolate...
in the medium, the glucosinolate content of the embryos declined, while in the presence of added glucosinolate, although no further increase was observed, the embryos maintained a 15- to 20-fold higher concentration of glucosinolate relative to the medium.

Uptake of benzylglucosinolate was not linear over the 26 h period investigated, although the deviation from linearity was slight (Fig. 4). The uptake showed a saturation effect with increasing substrate concentrations (Fig. 5). Depletion of substrate from the medium did not contribute to the saturation effect since no more than 5% of total glucosinolate originally in the medium could be accounted for in the embryos at any given concentration. Uptake of benzylglucosinolate was inhibited by CCCP, 2,4-DNP, and NaN₃ and was stimulated by Mg²⁺ (Table I). Whether the inhibitors were simply effective in killing the embryos or had more specific effects on transport of glucosinolate is not known.

Comparison of glucosinolate uptake by embryos excised at various stages of development with the glucosinolate content of embryos growing in situ showed that uptake in vitro rose rapidly from 16 to 20 DAP, peaked from 20 to 22 DAP, and declined slowly thereafter, exhibiting only residual activity at 36 DAP and beyond, while glucosinolate accumulation in

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**Figure 3.** Glucosinolate uptake (A) and fresh weight (B) of Golden rapeseed embryos removed at 24 DAP and cultured in vitro on Monnier’s medium. Embryos cultured in vitro in the presence of 1.0 mM 2-propenyl-glucosinolate (■), embryos transferred to fresh glucosinolate containing medium after 3 d in vitro (□), embryos transferred to fresh medium without glucosinolate after 3 d in vitro (△). Values are means of duplicate determinations; bars represent the range.

**Figure 4.** Time course for benzylglucosinolate uptake by Golden rapeseed embryos at 21 DAP. Initial glucosinolate concentration in the media was 4.0 mM. Values are means of duplicate determinations; bars represent the range.

**Figure 5.** Influence of benzylglucosinolate concentration on the uptake of benzylglucosinolate by Golden rapeseed embryos at 21 DAP. Values are means of duplicate determinations; bars represent the range.

**Table I.** Effect of CCCP, 2,4-DNP, NaN₃, and MgSO₄ on Benzylglucosinolate Uptake in Embryos 21 DAP

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Concentration</th>
<th>Relative Uptake of Glucosinolate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>CCCP</td>
<td>0.01</td>
<td>24.5 (2.4)</td>
</tr>
<tr>
<td>2,4-DNP</td>
<td>0.1</td>
<td>22.3 (0.6)</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>19.6 (3.2)</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td>100%</td>
</tr>
<tr>
<td>NaN₃</td>
<td>0.5</td>
<td>20.4 (2.2)</td>
</tr>
<tr>
<td>2.0</td>
<td>19.4 (2.8)</td>
<td></td>
</tr>
<tr>
<td>MgSO₄</td>
<td>2.0</td>
<td>191.8 (19.6)</td>
</tr>
</tbody>
</table>

* The incubation medium contained 4.0 mM benzylglucosinolate, 0.35 mM sucrose, 10.0 mM Mes/KOH buffer (pH 6.5), and 1% DMSO. The control rate was 0.57 (0.056) μmol g FW⁻¹ h⁻¹. * The incubation medium contained 4.0 mM benzylglucosinolate, 0.35 mM sucrose, and 10.0 mM Mes/KOH buffer (pH 6.5). The control rate was 0.36 (0.038) μmol g FW⁻¹ h⁻¹.
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Figure 6. Glucosinolate content in situ (○) and uptake in vitro using 1.0 mm 2-propenyl glucosinolate as a substrate (□) of Golden rapeseed embryos from 16 to 42 DAP. Values are means of duplicate determinations; bars represent the range.

situ commenced at 16 DAP, rose rapidly, then leveled off after 30 DAP (Fig. 6). Uptake in vitro, therefore, appears concomitant with glucosinolate accumulation in situ. The final glucosinolate content of embryos of B. napus cv Golden, 40 to 60 µmol (g FW)⁻¹, may depend on the position of the silique on the plant as has been observed for B. campestris (13).

The presence of glucosinolates in the seed of rapeseed affects the feeding value of the seed and of the meal after oil extraction. In addition to reducing palatability, nonruminant animals hydrolysis products can cause metabolic disorders, poor feed efficiencies, and reduced weight gain (1). To date, attempts to lower the glucosinolate content of rapeseed and other Brassicas by conventional sexual selection have resulted in an altered ability by the plant to synthesize glucosinolates (8). The results of the present study, along with the knowledge that embryo glucosinolate content is determined maternally (7, 9, 14), lead us to speculate that glucosinolates are accumulated in embryos in situ through an active transport process. Isolation by sexual breeding, in vitro cellular cultivation, or molecular genetics of rapeseed lines which differ in the ability of the maturing embryo to accumulate glucosinolates may be an alternate means of reducing seed glucosinolate content in this important oilseed crop.

LITERATURE CITED


