Characterization of Glucosinolate Uptake by Leaf Protoplasts of *Brassica napus*

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The uptake of radiolabeled *p*-hydroxybenzylglucosinolate (*p*-OHBG) by protoplasts isolated from leaves of *Brassica napus* was detected using silicone oil filtration technique. The uptake was pH-dependent with higher uptake rates at acidic pH. Imposition of a pH gradient (internal alkaline) across the plasma membrane resulted in a rapid uptake of *p*-OHBG, which was inhibited in the presence of carbonyl cyanide *m*-chlorophenylhydrazone, indicating that the uptake is dependent on a proton motive force. Dissipation of the internal positive membrane potential generated a small influx as compared with that seen for pH gradient (ΔpH). Kinetic studies demonstrated the presence of two uptake systems, a saturable and a linear component. The saturable kinetics indicated carrier-mediated translocation with a *Kₘ* of 1.0 mM and a *Vₘₐₓ* of 28.7 nmol/µL/h. The linear component had very low substrate affinity. The carrier-mediated transport had a temperature coefficient (*Qₐₚ*) of 1.8 ± 0.2 in the temperature range from 4–30 °C. The uptake was against a concentration gradient and was sensitive to protonophores, uncouplers, H⁺-ATPase inhibitors, and the sulphydryl group modifier *p*-chloromercuriphenylsulfonic acid. The carrier-mediated uptake system had high specificity for glucosinolates because glucosinolate degradation products, amino acids, sugars, or glutathione conjugates did not compete for *p*-OHBG uptake. Glucosinolates with different side chains were equally good competitors of *p*-OHBG uptake, which indicates that the uptake system has low specificity for the glucosinolate side chains. Our data provide the first evidence of an active transport of glucosinolates by a proton-coupled symporter in the plasma membrane of rape leaves.

Glucosinolates are amino acid-derived natural plant products containing a thioglucose and a sulfonated oxime. Glucosinolates are present in the Capparales order, including the family Brassicaceae, whose many cultivars have provided mankind with a source of condiments, vegetables, forage crops, and the economically important crop oilseed rape (*Brassica napus* L.). Glucosinolates are hydrolyzed by specific thioglucosidases, called myrosinases, to produce a wide range of degradation products, typically isothiocyanates, nitriles, and thiocyanates, with different biological activities. The glucosinolate/myrosinase system is believed to play a role in plant-pest interaction. The degradation products serve as attractants for insect specialists and as repellents for generalist herbivores, insects, and microorganisms (for review see Ref. 1).

Glucosinolates are present in all parts of the plant. The level of glucosinolates varies in different tissues at different developmental stages (2, 3) and is affected by external factors such as growth conditions (4, 5), wounding (6), fungal infection (7), actual and simulated insect damage (6, 8), and other forms of stress (9). Generally, high levels of biosynthesis are found in young leaves (10, 11), shoots, and silique walls (12); however, the high content of glucosinolates in young leaves declines rapidly after maturation (3). How plants manage to tissue-specifically and developmentally turn over the pool of glucosinolates in a physiological safe manner is not known.

There are several studies indicating that glucosinolates are transported within the plant. For example, in *Tropaeolum majus* high amount of benzylglucosinolate, which is primarily synthesized in the leaves, is also found to accumulate in other tissues, such as developing seeds, indicating translocation (11). Additionally, analysis of the glucosinolate profile of seed and leaf tissue of *B. napus* F1 hybrids, from reciprocal crosses between the cv. Cobra and a synthetic line, showed that the profile of the aliphatic glucosinolates in the seed was identical to the profile in the leaves of the maternal parent (13). This suggested that glucosinolate biosynthesis and glucosinolate interconversions (e.g. hydroxylation, desaturation, and alkenylation) did not take place within the embryo and that the fully formed glucosinolates were transferred from maternal tissue into the developing seeds. Furthermore, in vivo feeding of radiolabeled tyrosine to isolated seeds and intact siliques of *Sinapis alba* showed that although a low rate of *de novo* biosynthesis of *p*-OHBG occurred in the seed, the majority of *p*-OHBG was synthesized *de novo* in the silique wall and subsequently transported to the seed (14). Moreover, Toroser et al. (15) provided indications of the presence of *3′*-phospho-adenosine 5′-phosphosulfatedesulfoglucosinolate sulfotransferase activity in seeds of *B. napus*, which suggested that desulfoglucosinolates were the transport form of glucosinolates.

Recently, Brudenell et al. (16) have observed phloem mobility of radiolabeled glucosinolates and desulfoglucosinolates in *B. napus* plants. By comparison of the measured data with the predicted values obtained using the Kleier model for phloem mobility of xenobiotics, it was concluded that both glucosinolates and desulfoglucosinolates had suitable physicochemical properties to allow phloem mobility. In support of phloem mo-

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The abbreviations used are: *p*-OHBG, *p*-hydroxybenzylglucosinolate; DCCD, N,N′-dicyclohexylcarbodiimide; DES, diethylstilbestrol; CCCP, carbonyl cyanide *m*-chlorophenylhydrazone; PCMBs, *p*-chloromercuriphenylsulfonic acid; NBGS, *S*-propenylglutathione; TPP⁺, tetraphenylphosphonium chloride; MES, 4-morpholineethanesulfonic acid.
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bility of glucosinolates, aphid feeding experiments on black mustard, \( B. \) \( n \)igra \( L. \) cv. Koch, have shown that there was more than 10 m\( \mu \)mol of sinigrin in phloem sap of young leaves, whereas there was very low concentration of glucosinolates (about 1–2 m\( \mu \)mol) in mature, presenescent, and senescent leaves (17).

It has previously been shown that the uptake of glucosinolates in excised embryos of \( B. \) \( n \)apus exhibited saturable kinetics and was inhibited by protonophores such as CCCP, 2,4-dinitrophenol, and the respiratory chain inhibitor NaN\( \text{3} \) (18, 19). The data strongly indicated that the uptake of glucosinolates was by a carrier-mediated transport system. In the present paper, we have used isolated protoplasts from young leaves of \( B. \) \( n \)apus to study uptake of glucosinolates. Biochemical characterization of the uptake provided evidence for the presence of a glucosinolate/\( \text{H}^{+} \)-symporter in rape leaf cells.

**Experimental Procedures**

**Plant Material—Seeds of oilseed rape.** \( B. \) \( n \)apus \( L. \) (cv. Partim) were purchased from DLF Trifolium, Denmark. The seeds were soaked and sown in potting mix (Enhetssjö Kjörd, Weibulls, Sweden) with no application of nutrients. Plants were grown in the greenhouse for 25–28 days before leaves were harvested for protoplast preparation. The light intensity varied from 200 to 800 m\( \mu \)mol of photons/m\(^2\)/s during the 18-h photoperiod at 18–15 °C, respectively. The relative humidity was 50–65%, and water was supplied once every 2nd day. Tobacco plants (\( \text{Nicotiana tabacum} \), cv. Xanthi) were cultured on MS medium, pH 5.6 to 5.8 (20), containing 3% (w/v) sucrose, and 0.3% (w/v) \( \text{Difco} \) agar.

**Procedure of Preparation of Protoplasts.**—A Sep-p- \( \text{p} \) Pasteur pipette, diluted with W5 medium, centrifuged at 60 °C for 25 min, layer between the enzyme solution and W5 medium, were collected with uptake buffer (10 mM CaCl\( \text{2} \), 1 mM MgCl\( \text{2} \), 0.5 M sorbitol, and 10 mM \( \text{MES-KOH} \), pH 5.6 to 5.8). Free the protoplasts through the oil layer to the sorbitol layer while the free radiolabeled \( p \)-OHBG uptake solution (supernatant) remained on top of the hydrophobic mixture. The tips of the tubes containing the protoplast pellets were cut off and put into 2-ml Eppendorf tubes containing 0.2 ml of 1.5% (w/v) sodium dodecyl sulfate and sonicated for 10 min to rupture cell membranes and release radioactivity into the medium. At the same time, 1 \( \mu \)l of supernatant was also taken for radioactivity assay.

The radioactivity of the samples was determined by scintillation counting (Wallac 1400) after addition of 1.2 ml of scintillation fluid Ecoscint A. Uptake of \( p \)-OHBG was calculated as nanomoles of \( p \)-OHBG/\( \mu \)l of protoplast volume/h. The formula used was \( \text{cpm} \) in protoplast pellet/\( \text{H}^{2} \text{O} \) (cpm in the pellet) \( \times \) (cpm in 1 ml of supernatant/\( \text{H}^{2} \text{O} \) (dm) in 1 ml of protoplast-external p-OHBG/\( \text{H}^{2} \text{O} \) (dm) \( \times \) harvested protoplast volume (\( \mu \)l) and the amount of \( p \)-OHBG taken up (nanomoles). Correction for the \( p \)-OHBG present in the protoplasts after silicone filtration was done by subtracting the amount of \( p \)-OHBG in the protoplasts after silicone oil filtration. Correction was made by determining the uptake of \( p \)-OHBG adhering to the outside of the protoplasts was done by subtracting the amount of \( p \)-OHBG present in the protoplasts after silicone filtration following 30 s incubation in the uptake reaction mixture. The amount of \( \text{H}^{2} \text{O} \) in the protoplasts after silicone oil filtration did not change with time during the experimental period, indicating intactness of the protoplasts.

**Analysis of Temperature Optimum and pH Dependence of \( p \)-OHBG Uptake.**—The temperature optimum for uptake of \( p \)-OHBG by protoplasts of \( B. \) \( n \)apus was determined by incubation of 1 ml of protoplasts in standard uptake reaction mixtures at several temperatures ranging from 4 to 45 °C. The temperature coefficient (\( Q_{10} \)) for absorption was calculated according to the equation \( Q_{10} = \left( \text{rate at temperature } T_{2} / \text{rate at temperature } T_{1} \right)^{10} \), where \( T_{1} \) is the lower temperature and \( T_{2} \) is the higher temperature (26). The \( p \)-OHBG concentration (nmol/ml) was calculated as described above.

**Analysis of Substrate Specificity of the \( p \)-OHBG Transport Protein.**—The substrate specificity of the \( p \)-OHBG transport protein in protoplasts of \( B. \) \( n \)apus, with respect to other glucosinolates, was determined by competition experiments in which different glucosinolates at 1 or 5 times the concentration of radiolabeled \( p \)-OHBG were included in the uptake reaction mixture. Similarly, the affinity of the \( p \)-OHBG transport protein for other compounds such as glucosinolate degradation products, glutathione conjugates, hexoses, and amino acids was tested in standard uptake reaction mixtures. These competitors were present at 1 or 10 times the concentration of \( p \)-OHBG.

**Activators and Inhibitors of \( p \)-OHBG Uptake.**—The uptake of \( p \)-OHBG over the plasma membrane was characterized by preincubation of the protoplasts with various compounds for 30 min before the uptake reaction. The compounds used were: 2-(4-nitrophenyl)phenethylamine (22), 2-deoxy-D-glucose (23), 2-deoxy-d-glucose (24), 2-deoxy-d-glucose (25), and 2-deoxy-d-glucose (26). These compounds were preincubated with 5 \( \mu \)M of \( p \)-OHBG for 30 min at 25 °C to allow \( p \)-OHBG inside and outside the protoplasts to reach equilibrium, enabling estimation of the protoplast volume (exchangeable water volume) as described previously (23–25). In addition, the amount of \( \text{H}^{2} \text{O} \) in the cells was monitored by counting a small aliquot of samples at different time points to ensure that the cells were intact and stable during \( p \)-OHBG uptake. Uptake studies were carried out by addition of 2.5 \( \mu \)M of \( [1 \text{C}] \text{p-OHBG} \) (1.7 \( \mu \)mol) or [\( ^{3} \text{H} \text{p-OHBG} \) (0.16 \( \mu \)mol) to 1 ml of protoplasts (\( \times \)10\(^{6} \)) followed by incubation for different times and/or in the presence of different concentrations of substrates. Typical example of a uptake experiment was incubated at 25 °C for 15 min, following which 100-\( \mu \)l aliquots were removed from the uptake assay and transferred to 250-\( \mu \)l polyethylene microcentrifuge tubes (Carl-Ruth, Germany) containing a layer of 120 \( \mu \)l of hydrophobic mixture (silicone oil DC550/liquid paraffin, 21:4, Merck) on top of 10 \( \mu \)l of 0.5 M sorbitol. The aliquots were then counted in a liquid scintillation counter (Beckman LS-250, USA) after addition of 1 ml of scintillation fluid (Ecoscint A, Wallac 1400). The data strongly indicated that the uptake of glucosinolates was by a carrier-mediated transport system. In the present paper, we have used isolated protoplasts from young leaves of \( B. \) \( n \)apus to study uptake of glucosinolates. Biochemical characterization of the uptake provided evidence for the presence of a glucosinolate/\( \text{H}^{+} \)-symporter in rape leaf cells.
The concentration of 5 mM (Fig. 2). The kinetics were readily resolved into a saturable and a linear component by nonlinear regression to the two-component equation of Michaelis-Menten kinetics consisting of a saturable component and a linear component ($v = V_{\text{max}} [S]/(K_m + [S]) + k [S]$) using the SigmaPlot program. The kinetics consist of a saturable component and a linear component with the following parameter values: $V_{\text{max}} = 28.7 \text{ nmol/µl/h}$, $K_m = 1.0 \text{ mM}$, and $k = 0.9 \text{ nmol/µl/mM}$.

was kept at or below 0.1% (v/v), and controls were made in the corresponding ethanol concentration.

**Statistical Analysis**—Nonlinear regression (performed with the SigmaPlot curve fitter) was used to fit the kinetic data to the equation for two-component Michaelis-Menten kinetics consisting of a saturable component and a linear component ($v = V_{\text{max}} [S]/(K_m + [S]) + k [S]$). Statistical analysis (t test or analysis of variance) was performed using the SigmaStat program.

## RESULTS

**Uptake of p-OHBG by Protoplasts**—Protoplast yields per g fresh weight of *B. napus* and tobacco leaves were $1.9 \pm 10^9$ and $2.0 \times 10^9$, respectively. The viability of protoplasts as determined by neutral red uptake and Evans blue exclusion was $82.5 \pm 4.2\%$. Uptake of p-OHBG into *B. napus* protoplasts showed a steady increase within the 40 min investigated (Fig. 1). The concentration of p-OHBG in the cells after 10 min incubation with $[^3\text{H}]p$-OHBG was about $0.57 \text{ mM}$ which was much higher than that in the uptake medium (less than $0.16 \text{ mM}$). This shows that glucosinolates accumulate against a concentration gradient. Similarly, uptake of p-OHBG was detected in protoplasts isolated from hypocotyls and cotyledons of *B. napus* (data not shown). No p-OHBG uptake could be detected in tobacco leaf protoplasts (Fig. 1).

**Temperature Dependence of p-OHBG Uptake**—Uptake of p-OHBG was temperature-dependent with an optimum at 35 °C (data not shown). The temperature may influence the transport activity and/or the dissipation of the pH gradient. The average temperature coefficient ($Q_10$) determined from the data obtained between temperatures ranging from 4 to $30^\circ\text{C}$ was $1.8 \pm 0.2$, which indicates that active processes might be involved.

**Kinetics of Uptake**—When the uptake of p-OHBG by *B. napus* protoplasts was carried out in the presence of an increasing concentration of p-OHBG, the uptake kinetics were characterized by a non-saturating curve that approached linearity above the concentration of 5 mM (Fig. 2). The kinetics were readily resolved into a saturable and a linear component by nonlinear regression to the two-component equation of Michaelis-Menten kinetics using the SigmaPlot program. The saturable kinetics indicated carrier-mediated translocation with a $K_m$ of 1.0 mM and a $V_{\text{max}}$ of 28.7 nmol/µl/h. The linear component had very low substrate affinity and may be a channel, or an anion transport mechanism, on the plasma membrane (29).

**pH and Membrane Potential Dependence**—When uptake of p-OHBG by *B. napus* protoplasts was measured at different pH values, the rate of uptake increased with decreasing pH, with a maximum glucosinolate uptake at pH 4 (Fig. 3). The measured effect of lowering pH could reflect proton binding to the transporter and/or proton motive force. When CCCP, a protonophore, was added after 10 min of incubation, the uptake of p-OHBG stopped and the accumulated p-OHBG was released from protoplasts (Fig. 4). Transfer of protoplasts from pH 7 into pH 4 prior to uptake measurements resulted in a more than 3-fold increase in p-OHBG uptake (Fig. 5). These data are consistent with the idea that p-OHBG accumulation is dependent on proton motive force. Furthermore, we investigated whether there was an effect of altered membrane potential on p-OHBG uptake. Inside negative membrane potential can be decreased ($-\Delta\phi$) and increased ($+\Delta\phi$) by addition of SCN$^-$, TPP$^-$, or K$^+$, respectively (30–32). Treatment of protoplasts with 50 mM SCN$^-$ increased the rate of glucosinolate uptake by approximately 20%. Raising the concentration of K$^+$ or TPP$^-$ slightly reduced the rate of glucosinolate uptake (Fig. 5). The imposed $\Delta\phi$ effects on the uptake were very low, which indicates that the contribution of membrane potential to glucosinolate uptake is small.

**Specificity of the Uptake System**—The specificity of the p-OHBG uptake system was investigated by testing the uptake of several glucosinolates with different side chains, different glucosinolate degradation products, glutathione conjugates, sugars, glucoside, and amino acids, as potential substrate inhibitors or competitors of p-OHBG uptake. Competitive inhibition of p-OHBG uptake was only observed when different glucosinolates were included in the uptake reaction mixture (Fig. 6). No competitive inhibition was observed when glucosinolate degradation products, glutathione conjugates, sugars and dhurrin (a cyanogenic glucoside), and amino acids were included in the uptake reaction mixtures (Fig. 7). The equal ability of glucosinolates with structurally very different side chains to compete for uptake of radiolabeled p-OHBG indicates that many, if not all, glucosinolates share the same carrier.
Stimulation and Inhibition of the Uptake System—To characterize the mode of energization of the transport system, we studied the uptake of \( p \)-OHBG in leaf protoplasts in the presence of various compounds (Table I). A slight uptake stimulation of \( \sim 26\% \) was observed after the addition of fusicoccin (a toxin that activates plasma membrane \( H^+ \)-ATPase), and the \( H^+ \)-ATPase inhibitors \( Na_3VO_4 \), DCCD, and DES inhibited \( p \)-OHBG uptake. This indicates that the uptake is dependent on a functional \( H^+ \)-ATPase. Inhibitors associated with ATP metabolism such as arsenite, KCN, and NaN\(_3\) interfered with the uptake, which indicates that the uptake is dependent on energy. CCCP, which dissipates pH gradient, strongly reduced the transport, indicating that the uptake requires a proton motive force. The inhibitory effect of the impermeable sulfhydryl group modifier PCMBS on glucosinolate transporter was relieved by the inclusion of 5 mM DTT in the incubation medium.

DISCUSSION

In the present paper, we have measured uptake of radiolabeled \( p \)-OHBG in protoplasts isolated from leaves of \( B. \) napus using the silicone oil filtration technique (23, 24, 32). Biochemical characterization of the uptake indicates the presence of a glucosinolate/\( H^+ \) symporter.

Uptake of glucosinolates has previously been shown in vivo in excised embryos from \( B. \) napus (18, 19). In order to characterize the mechanism for glucosinolate transporter at the plasma membrane, we have developed a method for measuring uptake of glucosinolates in isolated leaf protoplasts. Compared to characterizing the mode of energization of the transport system, we studied the uptake of radiolabeled \( p \)-OHBG in leaf protoplasts in the presence of various compounds (Table I). A slight uptake stimulation of \( \sim 26\% \) was observed after the addition of fusicoccin (a toxin that activates plasma membrane \( H^+ \)-ATPase), and the \( H^+ \)-ATPase inhibitors \( Na_3VO_4 \), DCCD, and DES inhibited \( p \)-OHBG uptake. This indicates that the uptake is dependent on a functional \( H^+ \)-ATPase. Inhibitors associated with ATP metabolism such as arsenite, KCN, and NaN\(_3\) interfered with the uptake, which indicates that the uptake is dependent on energy. CCCP, which dissipates pH gradient, strongly reduced the transport, indicating that the uptake requires a proton motive force. The inhibitory effect of the impermeable sulfhydryl group modifier PCMBS on glucosinolate transporter was relieved by the inclusion of 5 mM DTT in the incubation medium.

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beled completed a minimum of four times. DTT, dithiothreitol. at 25 °C in the standard assay mixture, pH 5.5. Each measurement was then the uptake of p-OHBG (1.7 mol) in the presence of different compounds in concentrations corresponding to 1 or 10 times the concentration of [14C]-OHBG at 25 °C for 15 min and stopped by filtration through silicone oil.

Fig. 7. Study of competitive inhibition of uptake of radiolabeled p-OHBG by different compounds. 10٧ protoplasts in 10 mM MES-Tris uptake buffer, pH 5.5, were incubated with 2.5 μCi of [14C]-p-OHBG (1.7 μmol) in the presence of different compounds in concentrations corresponding to 1 or 10 times the concentration of [14C]-p-OHBG at 25 °C for 15 min and stopped by filtration through silicone oil. A, glucosinolate degradation products; B, glutathione conjugates; C, sugars and dhurrin (a cyanogenic glucoside); and D, amino acids. CK, control; P-ITC, phenylisothiocyanate; A-ITC, allylisothiocyanate; p-OHBOH, p-hydroxybenzyl alcohol; p-OHBCN, p-hydroxphenylacetonitrile; NBG, S-(p-nitrobenzyl)glutathione.

Table 1

| Treatment  | Concentration | p-OHBG uptake |
|-----------|---------------|---------------|
|            | ma | mol l µl h | % |
| Control    | 3.6 | 0.5 | 100 |
| Pasticcin  | 0.002 | 4.5 | 0.6 | 125.7 |
| NaN₃O₄     | 0.2 | 0.7 | 0.7 | 20.4 |
| DCCD       | 0.5 | 2.2 | 0.2 | 62.7 |
| DES         | 0.1 | 2.4 | 0.2 | 66.4 |
| Arsenite    | 0.5 | 0.9 | 0.1 | 23.3 |
| KCN         | 2   | 1.5 | 0.1 | 41.3 |
| NaN₃       | 2   | 1.9 | 0.3 | 52.7 |
| CCCP       | 0.01 | 0.9 | 0.2 | 25.1 |
| PCMBNS     | 1   | 0.6 | 0.3 | 15.6 |
| PCMBNS + DT  | 5  | 3.5 | 1.0 | 97.6 |

with intact tissues, protoplasts are single living cells where the plasma membrane is in direct contact with glucosinolates without any diffusional barriers. This allows for more rapid and sensitive kinetic studies as compared with studies using intact tissues. In comparison, the use of plasma membrane vesicles has the advantage of providing well defined membrane poten-
tials and pH gradients for electrophysiological studies, albeit in an in vitro system.

In the biochemical characterization of the glucosinolate transport system, we determined a high Q_{10} of 1.8 for p-OHBG accumulation. In the case of passive physical absorption, the Q_{10} value should be low, normally around 1.0 (26, 33). Theoretically, a solute must be neutralized and lipid-soluble in order to diffuse passively across a membrane. Due to the low pK_a value (~2) of the sulfonic acid group, glucosinolates invariably occur in nature in the anionic form (34). The presence of a glucose and a sulfonate moiety categorizes glucosinolates as hydrophilic, negatively charged compounds (34), which are unlikely to cross the plasma membrane by passive diffusion. This is consistent with the measured Q_{10} value. Furthermore, we observed that the internal concentration of p-OHBG in the protoplasts after 10 min of incubation was more than 3.6-fold higher than that in the reaction mixture (Fig. 1). This indicates that p-OHBG accumulates against a concentration gradient, as also evidenced by the decrease in uptake of p-OHBG by leaf protoplasts upon the addition of CCCP (Fig. 4). Moreover, a saturable component was identified at low glucosinolate concentrations. At high glucosinolate concentrations, we observed a linear low affinity component for rape leaf protoplasts (Fig. 2), which is in contrast to the saturation level reached in rapeseed embryos at 10 mM benzylglucosinolate (glucotropaeolin) (18). Similar nonsaturable components have been reported for sugar and amino acid uptake (32, 35–37). The linear component could be a carrier-mediated diffusion pathway, a non-carrier-mediated diffusion pathway, or possibly an unspecific amino acid transport protein on the plasma membrane (29).

With respect to the machinery for p-OHBG uptake, the pH dependence of uptake rates (Figs. 3 and 5), the abolishment of the pH dependence by administration of CCCP (Fig. 4 and Table 1), and the stimulation of uptake by H⁺-ATPase activator fusicoccin (Table 1) indicate that glucosinolates are absorbed in symport with protons. The membrane potentials artificially imposed upon the protoplasts by addition of TPP+ , K⁺, and NaSCN (30–32) contributed relatively little to the uptake of p-OHBG, which suggests that the glucosinolate uptake is not electrogenic and that the proton motive force is the primary energy source.

A range of inhibitors of H⁺-ATPase and ATP metabolism significantly reduced the uptake of p-OHBG by leaf protoplasts, which indicates that an active carrier is involved (Table 1). The inhibitory effect of PCMBNS on p-OHBG uptake is strong evidence for the involvement of a component of the plasma membrane (38, 39). Competition experiments with various compounds show that p-OHBG uptake activity is specific for glucosinolates (Figs. 6 and 7) and is independent of the structure of the different side chains (Fig. 6). This observation is consistent with previous studies on glucosinolate transport in vivo (16, 18, 19) and with in vitro studies on glucosinolate uptake with recombinant amino acid transporter and with recombinant hoxor transporters (data not shown). Accordingly, several transporters of major organic solute in the plasma membrane could be excluded as candidates for p-OHBG transport. This indicates that a specific glucosinolate carrier is responsible for intercellular glucosinolate transport. Interestingly, there was no detectable glucosinolate uptake activity in the non-glucosinolate plant tobacco (Fig. 1), which indicates that the existence of glucosinolate transporter(s) is specific for glucosinolate-producing plants.

The physiological role of a glucosinolate transporter in leaves of oilseed rape is unknown. A possible role could be to reabsorb glucosinolates that have leaked out of the cell, equivalent to the presence of sucrose transporters in all the cell types in source.
tissue (40). Alternatively, the glucosinolate transporter might play a role in translocation of glucosinolates in the plant. Phloem mobility of radioabeled glucosinolates has recently been demonstrated (16), suggesting that glucosinolate translocation may play an important role in glucosinolate metabolism.

In conclusion, we have provided biochemical evidence for the existence of a plasma membrane glucosinolate/H⁺ symporter. Cloning of the transporter is in progress, and will help to provide the necessary tools to address the question of where the transporter is expressed and what is the physiological function of the transporter. The biochemical characterization of the transporter facilitates identification at the molecular level, thus providing greater insight into the process of glucosinolate translocation in planta.

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