

Substrate specificity of the hexose carrier in the plasmalemma of *Chenopodium* suspension cells probed by transmembrane exchange diffusion*

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Abstract. Substrate specificity of the proton-driven hexose cotransport carrier in the plasmalemma of photoautotrophic suspension cells of *Chenopodium rubrum* L. has been studied through the short-term perturbation of ^{14}C -labelled efflux of 3-O-methyl-D-glucose. Efflux, occurring exclusively via carrier-mediated exchange diffusion, is *trans*-stimulated by the substrate and *trans*-inhibited by the glucose-transport inhibitors phlorizin ($K_i = 0.79 \text{ mM}$) and its aglucon phloretin ($K_i = 84 \mu\text{M}$); with both inhibitors, 3-O-methyl-D-glucose efflux may be blocked completely. *Trans*-stimulation of efflux (up to fourfold) by a variety of the D-enantiomers of neutral hexoses, including glucose ($K_i = 48 \mu\text{M}$), 3-O-methyl-D-glucose ($K_i = 139 \mu\text{M}$), and fructose ($K_i = 730 \mu\text{M}$), but not by, for instance, D-allose, and L-sorbose, shows that carrier-substrate interaction critically involves the axial position at C-1 and C-3, respectively. We suggest that substrate binding by the *Chenopodium* hexose carrier involves both hydrophobic interaction with the pyran-ring and hydrogen-ion bonding at C-1 and C-3 of the D-glucose conformation.

Key words: Cell culture (hexose carrier) - Carrier specificity - *Chenopodium* - Compartmental flux analysis - Hexose proton cotransport - Phlorizin

Introduction

Although the suspension cells of *Chenopodium rubrum* that we investigated have been propagated

* This work is derived from J. P. Gogarten's doctoral thesis

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Abbreviations and symbols. FW = fresh weight; 3-OMG = 3-O-methyl-D-glucopyranoside; subscripts C, O, V = cytoplasm, outside, vacuole; \mathcal{J}_{XY} (\mathcal{J}_{XY}^*) = unidirectional flux (of tracer) [$\text{mol min}^{-1} \text{g}_{\text{FW}}^{-1}$] from compartment X into Y; K_i = half-saturation constant [M]

photoautotrophically for over ten years (Hüsemann and Barz 1977), they still possess a proton-dependent hexose-uptake system (Gogarten and Bentrup 1984; Bentrup 1985). Apart from nutritional carbohydrate uptake, the presence of a hexose-transport system in photoautotrophic cells of a higher plant indicates a role in the phloem-bound long-distance transport of carbohydrates (compare Eschrich 1980; Gogarten 1986).

There are several difficulties in interpreting and comparing experimental results from studies of hexose transport that have been circumvented by the present study. The use of photoautotrophic cells obviates the need for a starvation period prior to the experiments, as the cells are cultured in a carbohydrate-free medium; thus influences by energy deprivation are less likely to occur. The use of suspension cells with cell walls only about $0.1 \mu\text{m}$ in thickness, whose transport-relevant properties are characterized in terms of charge density, porosity and hydrolytic enzymes (Gogarten 1988), favourably lends itself to control external sugar and ion concentrations at the membrane's transport sites. Thus, the use of suspension cells minimizes uncertainties arising from unstirred layers covering the transport sites, or from disrupted cells, as is invariably the case if tissues or protoplasts are used. The most pronounced experimental advantage, however, turned out to be the feasibility of short-time perturbation of a given steady-state efflux of labelled 3-O-methyl-D-glucose (3-OMG).

Efflux experiments have been a valuable tool in plant physiology, mainly for investigation of the ionic composition of cells and cell compartments by compartmental analysis (Walker and Pitman 1976). This technique has been extended to organic molecules, e.g. to abscisic acid in roots of *Hordeum distichon* (Behl et al. 1981) and guard cells of *Valerianella locusta* (Behl and Hartung 1986). We have

further developed this technique to quantify fluxes and compartmentation under net flux conditions as encountered, for instance, in studies using transport of non-metabolizable organic substrates like 3-OMG (Gogarten and Bentrup 1983). Our version of compartmental flux analysis has turned out to be a powerful tool in revealing subcellular hexose compartmentation and the kinetic properties of a putative hexose carrier at the plasmalemma of *Chenopodium* suspension cells. Explicitly, since labelled 3-OMG is taken up into the vacuole, a large-amplitude slow component of the efflux kinetics ($t_{1/2} \approx 5$ h) emerges which can be repeatedly and reproducibly perturbed by, and thus probes the operation of, the hexose carrier, because the cytoplasm is quickly replenished (and re-labelled) by the large vacuole (Gogarten and Bentrup 1983). A transient decrease in the rate of loss of radioactivity caused by filling of the cytosol with non-radiolabelled substrate was observed by Cram (1968) in the case of chloride transport in carrot.

We demonstrate that *trans*-stimulation and *trans*-inhibition by substrate and transport inhibitors, respectively, of 3-OMG efflux may reveal transport-relevant molecular details of the carrier's hexose-binding site (see Stein 1967; Sanders et al. 1984). A companion paper (Gogarten and Bentrup 1989) deals with the driving force and voltage dependence of this carrier.

Material and methods

Plant material. The photoautotrophic suspension culture of *Chenopodium rubrum* L. was established by Hüseemann and Barz (1977) and has been subcultured in our laboratory since 1979 (see Gogarten-Boeckels et al. 1985). Prior to experiments the suspension cells were incubated for at least 12 h in a test medium (preincubation medium) with the following composition: 2 mM NaCl, 0.1 mM KCl, 0.2 mM CaCl₂, 0.1 mM MgCl₂ and 2 mM NaH₂PO₄/Na₂HPO₄ buffer of pH 5.5. In this medium, at atmospheric CO₂ pressure, the cells retain their vitality for periods over 6 d as judged by the phenosafranine or fluorescein diacetate test (Gogarten-Boeckels et al. 1985) as well as by the transport properties of the plasmalemma. Cells were harvested by gravity filtration over a 15- μ m-mesh nylon net. One gram fresh weight (FW) of the harvested cells contains about 20–25 mg_{DW}, $5 \cdot 10^6$ – $10 \cdot 10^6$ cells, 0.05–0.1 m² cell surface, 0.5 ml vacuolar volume, 50 μ l cytoplasmic volume and approx. 250 μ g chlorophyll. For experiments conducted with cells in suspension, the cells were illuminated by light from white fluorescent lamps of 10 W m⁻²; the temperature was 24–26°C. Mixing was achieved by a suspended magnetic stirrer or round-shaker with a frequency 20% greater than that leading to turbulent flow.

Glucose concentrations were measured by the glucose-oxidase/peroxidase method as described by Bergmeyer and Brent (1970) with slightly different concentrations and reduced volumes. Concentrations of 3-OMG and other reducing sugars were de-

termined by an adaptation of the Somogyi method (Nelson 1944; Moore 1974).

Radioactivity was measured by liquid scintillation counting using Unisolve 100 (Zinsser, Frankfurt, FRG) in a betamatic scintillation counter (Kontron, Zürich, Switzerland). Quench correction was calculated using the channels-ratio method. Cells were diluted with scintillation cocktail until the counting efficiency was greater than 45% as calculated from the channel ratio and quench polynomial.

Metabolism of 3-OMG was measured as described in Gogarten and Bentrup (1983); after 6 d of incubation with [U-¹⁴C]-3-OMG, 70% of the incorporated radioactivity was recovered as 3-OMG, 8% comigrated with sugar phosphates and 15% comigrated with sucrose. The only substance identified in the exit medium (exit = efflux without 3-OMG added to the medium) was 3-OMG.

Efflux experiments. Cells were preincubated in a test medium with added [U-¹⁴C]-3-OMG. The cell density was 0.05–0.1 g_{FW} ml⁻¹; the specific activity was adjusted so that the cells had incorporated at least 30 MBq g_{FW}⁻¹ at the end of the loading period, corresponding to 33–270 MBq g_{FW}⁻¹ added to the incubation medium at the beginning. One g_{FW} of cells was transferred into an efflux chamber and, at the beginning of the efflux, the medium was perfused through the packed cells. The 3-OMG-concentration in this medium – henceforth called standard efflux medium – was the same as in the preincubation medium at the end of the loading period without radioactive label. The efflux chamber was made from Plexiglas and was similar to a short chromatography column (1.7 cm diameter, approx. 2.5-cm high). The bottom consisted of a porcelain frit covered with a 15- μ m-mesh nylon net. The cells were packed by gravity and by flow of the medium (about 1 ml min⁻¹). The packed cells were covered with another circular nylon net to achieve a uniform flow washing the cells. A head of about 1 ml solution was kept above the cells. In order to exclude non-mixed spaces the washing solution (efflux medium) was added dropwise. The kinetics of the chamber (i.e. the exchange of tracer in the external medium) yielded a time constant of about 1 min. The efflux medium was collected by a fraction collector in 1- to 5-min intervals. The radioactivity released from the cells was measured by liquid scintillation counting.

Compartmental analysis. The first 120 min of the efflux kinetics were selected for analysis. The data were either analyzed as described by Gogarten and Bentrup (1983) or the parametrised data were directly fitted by the pertinent nonlinear, nonautonomous set of differential equations as described in Gogarten (1986). All calculations were done on a Hewlett Packard (Bad Homburg, FRG) 9845B desktop calculator. A given perturbation of the steady-state flux, i.e. of the slow component of the efflux kinetics when the cytoplasm gains as much radioactivity from the vacuole as it loses to the apoplast, was evaluated according to the following reasoning: immediately after the flux across the plasmalemma, \bar{O}_{CO} , changes (perturbed flux $\bar{O}_{CO,d}$), the cytoplasmic specific activity, s_{CO} , is still unchanged; the changed flux thus is directly mirrored by the relative change in radioactivity release

$$\frac{\bar{O}_{CO,d} - \bar{O}_{CO}}{\bar{O}_{CO}} = \frac{(\bar{O}_{CO,d} - \bar{O}_{CO}) s_{CO}}{\bar{O}_{CO} s_{CO}} = \frac{\bar{O}_{CO,d} - \bar{O}_{CO}}{\bar{O}_{CO}}$$

where \bar{O}_{CO} and $\bar{O}_{CO,d}$ denote the unidirectional fluxes of 3-OMG and of radioactivity, respectively, from the cytoplasm to the outside; s_{CO} stands for the actual specific radioactivity in the cytoplasm and the index d denotes the same quantities immedi-

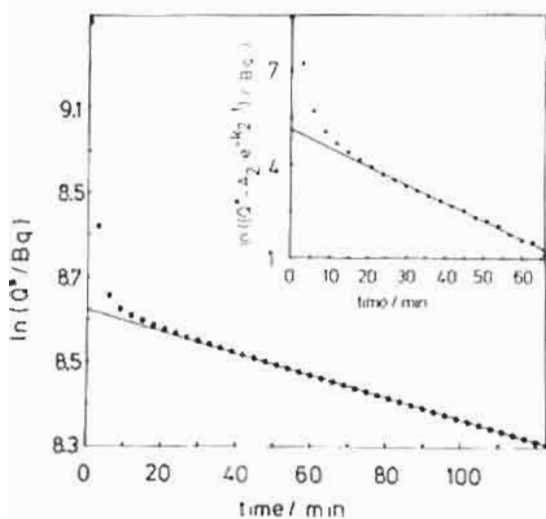


Fig. 1. Representative kinetics of ^{14}C -labelled 3-OMG efflux: semilogarithmic plot of the radioactivity contents Q^* of the efflux chamber holding a *Chenopodium* cell suspension. By standard efflux analysis two flux components may be extracted (Gogarten and Bentrup 1983); i.e. subtraction of the exponential function describing the slow component, $[A_{2m} = A_2 \exp(t/\tau_2)]$, yields a second, faster component (inset) attributed to the efflux across the plasmalemma, Φ_{CO} . Loading conditions: one g_{FW} in 20 ml test medium with initially 10 mM 3-OMG declining to 8.5 mM at the end of loading (24 h). Fluxes and concentrations have been calculated from the volume estimates given in *Material and methods* and the equations given in Gogarten and Bentrup (1983): $c_c = 90$ mM 3-OMG; $c_v = 55$ mM 3-OMG; $\Phi_{CO} = 370$ and $\Phi_{VC} = 62$ nmol 3-OMG $\text{min}^{-1} \cdot g_{FW}^{-1}$. c_c , c_v = cytoplasmic and vacuolar concentrations; Φ_{CO} , Φ_{VC} = unidirectional fluxes from cytoplasm to outside, and vacuole to cytoplasm.

ately after onset of perturbation. Note that after prolonged perturbation this equation does not hold; rather, radioactivity release then approaches again the steady-state net flux of radioactivity across the tonoplast, $\Phi_{VC} - \Phi_{CV}$, prior to the perturbation and, in fact, is independent of the fluxes across the plasmalemma.

Kinetics. All concentration-dependent kinetics were analyzed routinely with plots according to Lineweaver-Burk, Eadie-Hofstee and Hanes. The standard errors of the half-saturation constants and maximal effects were determined according to the iterative least-squares fit algorithm given by Wilkinson (1961).

Results

The addition of hexoses to the external medium induces several measurable responses from the suspension cells. The uptake of hexoses can be described by a single hyperbolic relation half-saturating at 60 μM D-glucose (see Bentrup 1985). In the measured concentration range (10 μM to 10 mM) we found no indication of a second (parallel) component or a diffusion resistance in series with the plasmalemma (unstirred layer, cell wall). When the

Table 1. Concentrations of 3-OMG in *Chenopodium* suspension cells, calculated from seven representative efflux experiments according to Gogarten and Bentrup (1983) and the volume estimates given in *Material and methods*. Efflux conditions: 17–36 h loading time with a range from 50 μM to 10 mM 3-OMG at the onset of loading. By uptake this initial external concentration (c_0) decreased to the c_0 values given in the first column. When only two components of the efflux kinetics (cuvette and cells) could be resolved, only the mean intracellular concentration is given. c_c , c_v = concentrations in cytoplasmic and vacuolar compartments

3-OMG concentration (mM)			
c_0	c_c	c_v	c_c/c_0
0.0015		0.79	527
0.015	2.3	7	153
0.057	10	27	175
0.075	12	18	160
0.15		26	173
1.09	56	25	51
8.2	71	53	8.6

data were analyzed by least-squares fit (Marquardt 1963) to the pertinent equations (Hill-Whittingham equation in the case of a series resistance, e.g. see Smith and Walker 1980), the least-squares error due to an increased number of parameters improved only insignificantly, i.e. <10%.

The 3-OMG efflux kinetics from *Chenopodium* suspension cells show two components that can be ascribed to intracellular pools of 3-OMG (Fig. 1). The two compartments giving rise to the two distinct components are identified as cytoplasm and vacuole, because, firstly, efflux-perturbation experiments show these two pools to be in series and, secondly, because of the amount of 3-OMG stored in the innermost compartment (Tables 1, 2).

Efflux experiments after varying loading conditions (Table 1) show that accumulation mainly occurs across the plasmalemma and that the accumulation ratio decreases with increasing concentration difference. Table 2 gives data from a batch of cells after different loading times in a high (10 mM) external 3-OMG concentration. Obviously, the amount of 3-OMG in the cytoplasm rises rapidly at the beginning of the loading period (0–1.33 h); 3-OMG taken up thereafter being mainly transported into the vacuole.

If, during the slow component of an efflux experiment, the 3-OMG concentration is changed (Fig. 2), increased 3-OMG concentrations lead to an increased loss of radioactivity; this can be attributed to an increased exchange by diffusion across the plasmalemma. The increased loss of radioactivity upon stimulation is far in excess of that

Table 2. Flux rate constants (k_1, k_2) flux components (A_1, A_2), cytoplasmic (c_c) and vacuolar (c_v) contents and ratios, respectively, and fluxes Φ for different loading times (t_{in} ; first column) in 10 mM 3-OMG (initial concentration). Experimental data for $t > 20$ min after start of efflux were parametricised by a least-squares fit of two exponentials, $A_1 \exp(k_1 t) + A_2 \exp(k_2 t)$. In an iterative algorithm this set of parameters was then used to find those numbers ($c_c, c_v, \Phi_{CO}, \Phi_{VC}$) which yielded the same two exponential functions as the experimental data. The residuals after fitting the experimental data and the data from the last simulation were nearly identical. For a detailed description of the algorithm see Gogarten and Bentrup (1983), and Gogarten (1986). c_0 = external concentration; Φ_{CO}, Φ_{VC} = unidirectional fluxes from outside to cytoplasm, and vacuole to cytoplasm; $\Phi_{CV} = \Phi_{VC}$ = net steady-state flux across the tonoplast

t_{in} (h)	k_1 (10^{-3} min^{-1})	k_2	A_1	A_2	[3-OMG] (mM)					Φ_{CO}	Φ_{VC}	$(\Phi_{CV} - \Phi_{VC})$
					c_0	c_c	c_v	c_c/c_0	c_v/c_c			
1.33	56	3.3	0.27	0.43	9.8	62	5	6.3	0.08	216	29	40
3.58	42	2.7	0.20	1.43	9.5	64	18	6.7	0.28	130	58	30
6.25	60	2.5	0.27	1.90	9.0	70	25	7.8	0.36	190	47	16
8.58	54	2.7	0.35	2.24	8.6	92	30	10.7	0.33	200	58	12
24.5	84	1.9	0.38	4.02	8.4	94	56	11.2	0.60	260	70	12

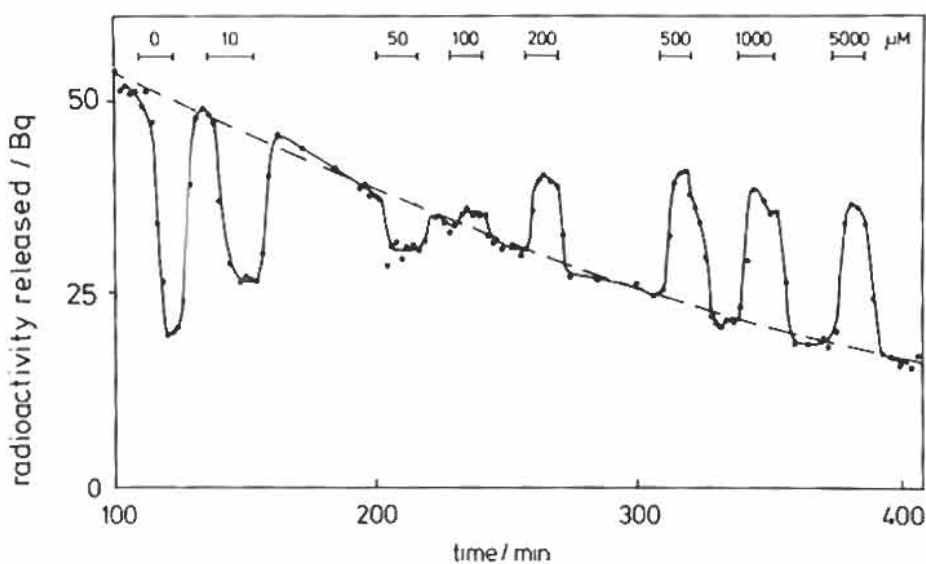


Fig. 2. Efflux kinetics showing *trans*-stimulation of 3-OMG efflux from *Chenopodium* suspension cells by the external 3-OMG. During the marked time intervals, 3-OMG in the efflux medium was temporarily shifted from the standard concentration of 78 μM to the indicated values

recycled by re-uptake during unstimulated efflux. Measurement of re-uptake by varying the rate of efflux-chamber perfusion, that is, varying the concentration of radioactivity in the external medium without changing the 3-OMG concentration, showed that 100% *cis*-inhibition of re-uptake would raise net radioactivity release by less than 21%.

Instead of varying the 3-OMG concentration in the efflux medium, other hexoses can be substituted for 3-OMG and obviously stimulate efflux of labelled 3-OMG to varying degrees (Fig. 3). Alternatively, if the efflux is conducted with nonsaturating external 3-OMG concentrations, other sugars can be added to the standard efflux medium: Figure 4 shows that sorbitol and L-sorbose,

in contrast to D-fructose, fail to stimulate 3-OMG efflux to a comparable degree.

A given substance added to the efflux medium may act in a number of ways. Firstly, the substance may be recognized and transported by the hexose carrier, leading to *trans*-stimulation of 3-OMG efflux (see Fig. 2). On the level of reaction kinetics *trans*-stimulation of a given efflux is plausible if immediate transmembrane recycling of the empty binding site to the cytosolic side takes more time than its recycling via three minimum steps of (i) substrate loading on the apoplasmic side, (ii) its subsequent translocation (probably only a conformational change in the carrier molecule allowing substrate access) to the other side, and (iii) unloading of the unlabelled substrate.

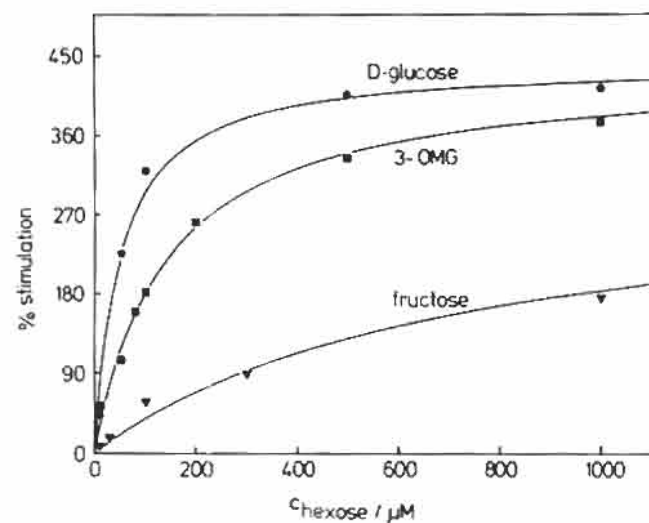


Fig. 3. *Trans*-stimulation of 3-OMG efflux from *Chenopodium* suspension cells by 3-OMG, D-glucose and D-fructose (compare Fig. 7). Stimulation is given relative to the flux across the plasmalemma (O_{CO}) without hexoses in the external medium. Least-squares fit after Wilkinson (1961) yielded (\pm SE): D-glucose: $K_1 = 48 (\pm 8) \mu\text{M}$; max. = $441 (\pm 14)\%$; 3-OMG: $K_1 = 139 (\pm 9) \mu\text{M}$; max. = $434 (\pm 7)\%$; D-fructose: $K_1 = 730 (\pm 140) \mu\text{M}$; max. = $316 (\pm 22)\%$.

Secondly, the substance may bind to the transporter, but not be translocated and released on the inside. This *trans*-inhibition of 3-OMG efflux is demonstrated for phlorizin and its aglucon phloretin: Fig. 5 shows efflux inhibition by phlorizin, and Fig. 6 gives data from the equivalent experiment with phloretin. With both substances, *trans*-inhibition of 3-OMG efflux may be extrapolated to 100% (compare inset of Fig. 6). Inhibition half-saturates at $790 \mu\text{M}$ phlorizin and $84 \mu\text{M}$ phloretin.

Thirdly, a given substance may show no or little direct interaction with the 3-OMG transport: radioactivity release is changed by less than 15%, as shown in Fig. 4 with sorbitol and L-sorbose. The marginal case of a substrate that is bound and transported via the transport system, but at such a slow rate that the binding site with that substrate bound is recycled as slowly as the empty binding site, may be subsumed also under this category, because such a substrate fits less well than D-glucose or 3-OMG (which both lead to a substantial increase in the recycling of the substrate-binding site; compare category no. 1).

Figure 7 shows structures of tested sugars and related compounds belonging to the first and the third transport category. Other tested compounds of no or little effect at 2 mM concentration include

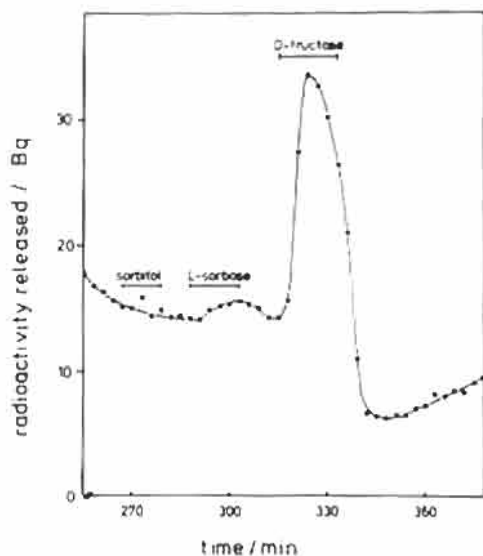


Fig. 4. *Trans*-stimulation of 3-OMG-efflux from *Chenopodium* suspension cells by addition of 2 mM D-fructose, L-sorbose, and sorbitol, respectively, over the indicated time intervals. The standard efflux medium contained $160 \mu\text{M}$ 3-OMG. Note the rise of radioactivity release after return to the standard efflux medium as a result of relabelling of the cytoplasm from the vacuole.

open-chain sugar alcohols (mannitol, L-sorbitol = D-glucitol) or cyclic sugar alcohols (*myo*-inositol), but also L-glucose and disaccharides (maltose, cellobiose). To our surprise, however, glucose-6-phosphate and sucrose also stimulated 3-OMG efflux. It turned out that both are hydrolyzed by cell-wall-bound enzymes, whereas fructose remained unmodified in the external medium (data not shown).

Addition of α - and β -D-glucose to the standard efflux medium ($150 \mu\text{M}$ 3-OMG, pH 6.5), dissolved immediately before addition, produced nearly identical stimulating effects (α - gave +114% and β -D-glucose +118% relative to standard efflux medium).

The effect of the charged sugar derivatives glucosamine (i.e. 2-amino-2-deoxy-D-glucose) and glucuronic acid on 3-OMG efflux became more evident at pHs favouring the neutral form; 2 mM glucuronic acid stimulated at pH 4.5, whereas no effect was observed at pH 6.5. With glucosamine, the k_1 of stimulation decreased from about 4.4 mM glucosamine at pH 5.5 to $550 \mu\text{M}$ at pH 6.5, whereas the concentration dependence of *trans*-stimulation by external 3-OMG was only little affected by the shift of external pH from 5.5 towards 6.5.

Substrates of other H^+ -cotransport systems like α -aminoisobutyric acid had no effect on 3-

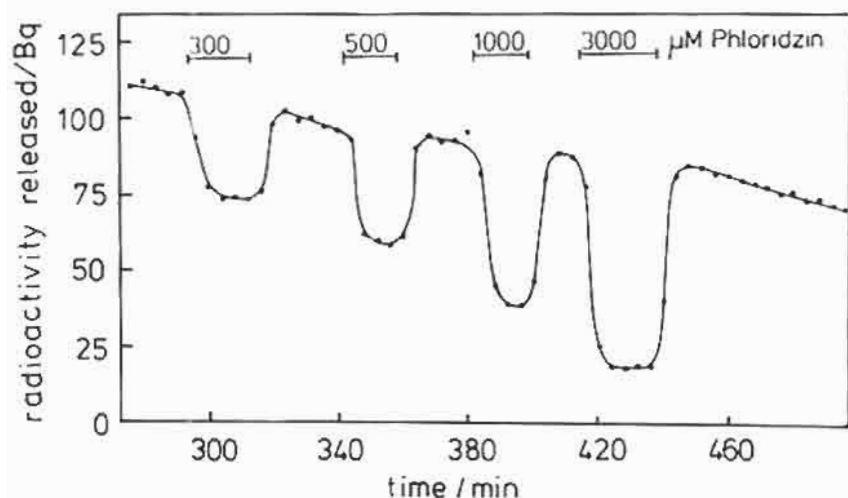


Fig. 5. Efflux kinetics of 3-OMG as in Figs. 2 and 4. During the marked periods the given concentration of phlorizidin (and 92 μM 3-OMG) was present in the efflux medium. The data may be replotted to show that inhibition saturates near 100% and half-saturates at 0.79 mM phlorizidin (compare Fig. 6)

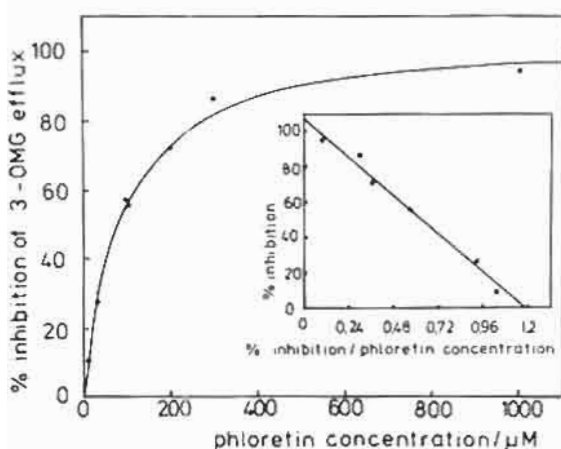


Fig. 6. Inhibition of 3-OMG efflux from *Chenopodium* cells as a function of external phloretin. (Same experimental procedure as shown in Fig. 5 for phlorizidin). The inset Eadie-Hofstee plot yields a K_i of 84 (± 7) μM phloretin, and maximal inhibition of 105 (± 3)%

OMG efflux when added to the efflux medium at 2 mM concentration (k_1 for α -aminoisobutyric acid uptake in *Chenopodium* is about 10 μM ; data not shown).

Discussion

Our compartmental analysis of 3-OMG-efflux experiments shows that 3-OMG, being offered for prolonged times, is mainly transported across the tonoplast into the vacuole (Table 1). The maximal fluxes across the tonoplast are about one-third of that determined for the plasmalemma, i.e. approx. 60 versus 200 $\text{nmol}\cdot\text{min}^{-1}\cdot\text{g}_{\text{FW}}^{-1}$ (Table 2). The possibility that the innermost compartment (i.e. the compartment from which the radioactivity con-

tent of the cytoplasm is refilled after a transient *trans*-stimulation of efflux) may be attributed to a metabolic (possibly cytosolic) pool, instead of the vacuole, can be excluded, since the major fraction (>95%) of the incorporated radioactivity undergoes the efflux and is attributable to the slowly exchanging compartment, whereas the metabolized fraction of labelled 3-OMG was rather small (20% after 6 d).

Trans-stimulation of efflux by external substrate and, vice versa, of influx by internal substrate is an excellent indicator of interaction between a transported substrate and a given carrier having a single binding site (compare Stein 1967). In lower plants, evidence for such a hexose carrier has been found in *Chlorella* (Komor et al. 1972) and the liverwort *Riccia fluitans* (Gogarten and Bentrup 1983). Our data infer the following molecular features for the carrier in the *Chenopodium* plasmalemma. In Fig. 8, we have outlined a clearly speculative model of the carrier binding site. Firstly, any transportable substrate must be able to form a pyran-ring. Secondly, the only hydroxyl group strictly preventing transport if oriented axially (i.e. normal to the plane of the pyran-ring), was the one at C-3 (D-allose and D-ribose; Fig. 7). Since, on the other hand, 2-deoxy-D-glucose was transported at least as well as D-glucose, the hydrophobic area of the D-glucose molecule below its equatorial plane, especially below C-2 and C-3, seems to be essential for substrate binding. Hydroxyl groups pointing axially above the ring plane apparently do not affect binding, nor is the CH_2OH -group at C-6 required for transport.

Thirdly, glucosamine is effectively transported by the 3-OMG-transporting system of *Chenopodium* suspension cells, but only in its neutral form

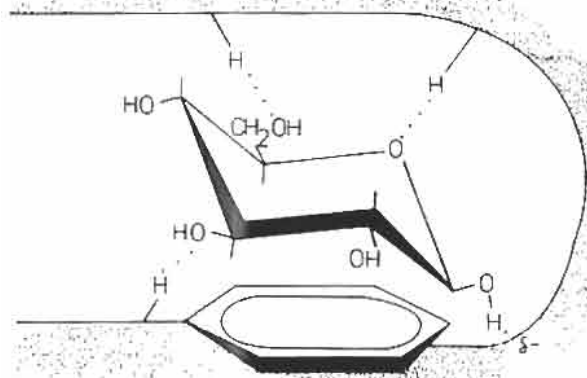


Fig. 8. Schematic view of the interactions between substrate and substrate-binding site proposed for the hypothetical hexose transporter in the plasmalemma of *Chenopodium*. Dots represent hydrogen bonding, the phenyl residue symbolizes hydrophobic interaction between binding-site and the hexose pyran ring, and δ^- the transporter's negative charge (see text)

fructose (equivalent to C-5 in D-glucose) pointing axially downwards in Fig. 7 may bend slightly to the equatorial plane, so the chair conformation becomes a little more planar. In L-sorbose this induced fit might fail because deformation towards a more planar structure would shift the equatorial hydroxyl at C-5 (equivalent to C-2 in D-glucose) somewhat below the equatorial plane and thus impede the postulated hydrophobic interaction (Fig. 8).

Finally, we briefly consider this hydrophobic interaction. The depicted model of an arbitrary phenyl residue (methyl or indole groups would be fairly equivalent) and four hydrogen bonds in a plane including angles of about 120 and 60 degrees, respectively, is consistent with all observations of this study. Moreover, the hydrophobic interaction nicely explains the strong (up to 100%) inhibition of the *Chenopodium* hexose transporter by phlorizin and phloretin (Fig. 5, 6). In plants, so far, inhibition of a hexose carrier by phlorizin has been found in *Riccia fluitans* by Felle et al. (1983). The unexpected finding that phloretin, the aromatic aglucon of phlorizin, is the stronger inhibitor, clearly points to an interaction of its 2,4,6-trihydroxy-acetophenone group with the hexose-binding site. Actually, 0.1 mM 2,4,6-trihydroxy-acetophenone significantly inhibits 3-OMG efflux (data not shown). In a parallel study, hydrophobic interaction has been postulated to be involved in substrate recognition by a sucrose carrier in protoplasts derived from soybean cotyledons (Hitz et al. 1986).

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