

Functional expression of a plant plasma membrane transporter in *Xenopus* oocytes

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A full-length cDNA clone for the H⁺/hexose co-transporter (STP1) from *Arabidopsis thaliana* has been transcribed in vitro and the mRNA injected into *Xenopus* oocytes. Under optimized conditions, oocytes injected with the STP1 mRNA accumulated 3-O-[methyl-¹⁴C]glucose at rates of more than a 1000-fold greater than water-injected control oocytes. A hexose-elicited depolarization of the oocyte membrane potential was demonstrated, and uptake was shown to be stimulated by low external pH, confirming the activity of a H⁺/hexose co-transport system. This is the first example of the functional expression of a plant membrane transporter in oocytes.

Arabidopsis thaliana; Hexose transporter; Proton symporter; Heterologous expression; *Xenopus* oocyte

1. INTRODUCTION

Oocytes from *Xenopus laevis* have been used extensively for the heterologous expression of mammalian transport systems [1] and have proved to be an important tool for characterization of the transporters, and, in some instances, for isolation of the corresponding genes [2]. Although a yeast membrane receptor protein has been expressed successfully in *Xenopus* oocytes [3] there have been no reports of the functional expression of a transport protein from a higher plant. However, a number of studies have demonstrated that plant mRNAs can be translated in *Xenopus* oocytes (e.g. [4,5]), and that, where appropriate, the encoded plant proteins can be successfully glycosylated and secreted [6,7].

Recently, cDNA clones for H⁺/hexose symporters have been isolated from both *Chlorella kessleri* and *Arabidopsis thaliana* and their identities confirmed by heterologous expression in the fission yeast *Schizosaccharomyces pombe* [8,9]. Here we report the functional expression of the *Arabidopsis* H⁺/hexose co-transporter (STP1) in *Xenopus* oocytes.

2. MATERIALS AND METHODS

2.1. Synthesis of STP1 mRNA

A full-length cDNA for the *Arabidopsis* STP1 H⁺/hexose co-transporter (pTF414A) was kindly provided by Dr. N. Sauer. The 1,800 bp insert was subcloned into the transcription vector pGEM-3Z

Abbreviations: 3-OMG, 3-O-methylglucose; MBS, modified Barth's saline; SDS, sodium dodecyl sulphate.

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(Promega Corporation, Madison, WI, USA) to give pAGT1, and the orientation and identity of the sequence was confirmed by partial sequence analysis. For in vitro transcription, pAGT1 was linearized with *Hind*III, and mRNA synthesized with T7 RNA polymerase in the presence of a cap analogue (GpppG).

2.2. Oocyte injections

Oocytes were surgically removed from adult female *Xenopus laevis* (Blades Biological, Edenbridge, Kent, UK) and follicular cells surrounding the oocytes were removed by treatment with collagenase (Sigma Chemicals, Poole, Dorset, UK; type 1A) at 2 mg/ml in modified Barth's saline (MBS) [10] for 2 h. MBS contained 88 mM NaCl, 1 mM KCl, 0.82 mM MgSO₄, 0.41 mM CaCl₂, 0.33 mM KNO₃, 2.4 mM NaHCO₃, and 15 mM HEPES adjusted to pH 6 with HCl. After thorough washing with MBS, the oocytes were kept in MBS supplemented with streptomycin and penicillin, both at 10 µg/ml, for 12–24 h at 18°C. Healthy looking stage V–VI oocytes [11] were injected with 50 ng of mRNA (determined by absorbance at 260 nm) in 50 nl water or with 50 nl of water alone using a glass capillary attached to a semi-automated microinjector (Drummond Scientific Co., 500 Parkway, Broomall, PA, USA). The injected oocytes were incubated at 18°C in MBS for up to 5 days. The protein synthetic ability of each batch of oocytes was tested by injecting a number of the oocytes with synthetic human α -amylase mRNA and assaying for secreted α -amylase enzyme activity after 24 h [12].

2.3. Hexose uptake assay

Functional expression of the hexose transporter was assayed by the uptake of 3-O-[methyl-¹⁴C]glucose (3-OMG; Amersham International, Amersham, UK). Unless stated otherwise, uptake was assayed at 22°C in MBS, pH 6, without antibiotics and with an external 3-OMG concentration of 30 µM (specific activity 4.62 MBq/µmol). Oocytes were incubated in radiolabelled hexose for 30 min and then rinsed five times with ice-cold unlabelled MBS. Individual oocytes were then lysed by incubating in 0.5 ml 1% SDS (v/v) for 1 h at 20°C.

2.4. Oocyte volume measurements

Oocyte size was measured using a microscope fitted with a long working distance x4 objective lens, a x8 ocular lens, and a video camera. The mean oocyte diameter was measured by image analysis of the video camera output (Magiscan 2A, Joyce Loebel, Team Valley, Gateshead, UK). Oocyte volume changes were then measured after an

osmotic challenge in which the bathing solution was replaced with 50% (v/v) hypotonic Barth's saline [13].

2.5. Oocyte membrane potential measurements

Standard microelectrode techniques were used to measure the oocyte membrane potentials and output voltages from an electrometer (WP Instruments, New Haven, CT, USA; model FD 223) were recorded at a sampling frequency of 10 Hz on an Opus PC V microcomputer using software developed by I.R. Jennings (University of York, UK) [14]. Microelectrodes were filled with 100 mM KCl and had resistances in the range 5–10 M Ω when measured in 100 mM KCl. Throughout the recordings oocytes were continuously superfused with MBS.

3. RESULTS AND DISCUSSION

Fig. 1 shows the time-course for expression of hexose uptake activity in *Xenopus* oocytes injected with synthetic mRNA encoding the *Arabidopsis* STP1 hexose transporter. Oocytes injected with STP1 mRNA showed a high rate of radiolabelled 3-OMG uptake compared to water-injected controls. Individual batches of *Xenopus* oocytes differed markedly in their endogenous rates of 3-OMG uptake, with uptake rates by water-injected controls varying from 0.2 to 80 fmol/oocyte/min at 22°C in MBS, pH 6. However, the endogenous rates were always small when compared with oocytes injected with STP1 mRNA. In the experiment shown in Fig. 1, the highest rates of 3-OMG uptake were obtained 3–4 days after STP1 mRNA injection, when the rate was 3,000-fold greater than the water-injected control. A similar time-course was observed for expression of barley α -amylase mRNA in oocytes [15].

Fig. 2 shows how substrate concentration affected the uptake of 3-OMG by oocytes injected with STP1 mRNA. The kinetics confirmed to a Michaelis-Menten

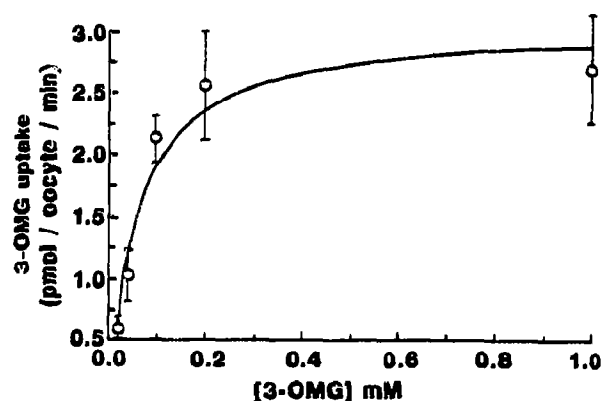


Fig. 2. The relationship between 3-OMG concentration and the rate of 3-OMG uptake by oocytes injected with STP1 mRNA. Each value is the mean \pm S.E.M. of 10 oocytes. The curve is a fitted Michaelis-Menten function with a K_m of 60 μ M and a V_{max} of 3 pmol/oocyte/min.

function with a K_m for 3-OMG of 100 μ M at pH 6. This compares well with the K_m of 60 μ M obtained at the same pH for the STP1 transporter expressed in *S. pombe* [9].

By incubating oocytes in radiolabelled 3-OMG for 24 h, measuring the diameter of oocytes (means 1.23 ± 0.15 mm) and assuming a spherical volume, the accumulation of the non-metabolisable hexose can be calculated. After 24 h in a solution containing 30 μ M 3-OMG, the mean (\pm S.E.M.) internal 3-OMG concentration in ten water-injected control oocytes was 7.4 (\pm 1.3) μ M. In the same experiment, ten oocytes injected with STP1 mRNA accumulated 3-OMG to a mean concentration of 131 (\pm 15.4) μ M, over 4-fold higher than the external

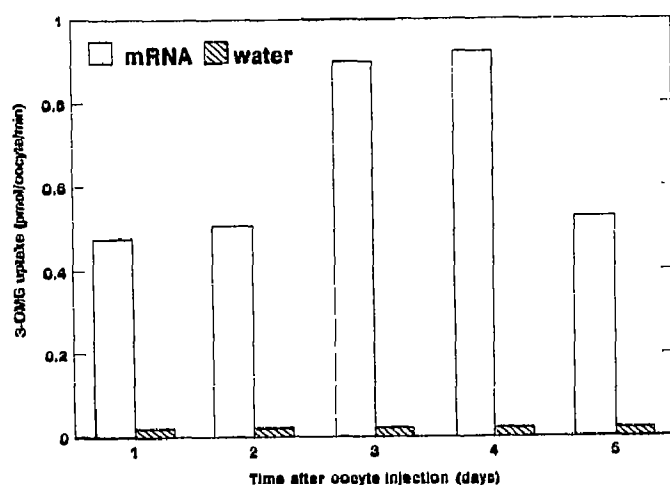


Fig. 1. Time-course of expression of hexose transporter activity in oocytes injected with STP1 mRNA. The oocytes were injected with 50 ng mRNA or with water at day 0 and incubated in MBS at 18°C. At each time point, 10 oocytes were transferred to MBS containing 14 C-labelled 3-OMG for measurement of uptake rates. Standard errors of the mean are indicated for each value.

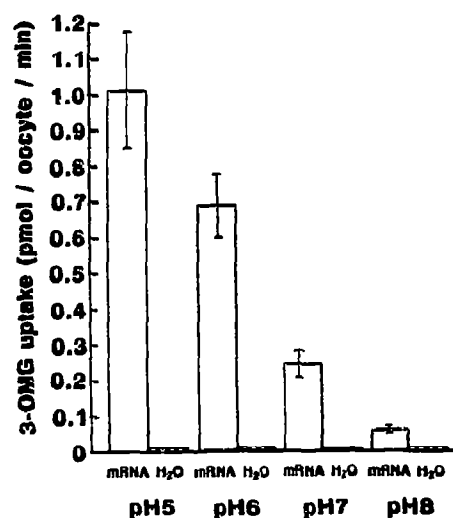


Fig. 3. The effect of external pH on 3-OMG uptake by oocytes injected with STP1 mRNA or with water. The pH of MBS was modified by the addition of HCl or NaOH and oocytes were washed twice in the modified uptake solution before incubation for 30 min in the same solution containing 14 C-labelled 3-OMG. At the end of the incubation the pH of the bathing solution was tested with a pH electrode and found to be unchanged. Each value is the mean \pm S.E.M. of 10 oocytes.

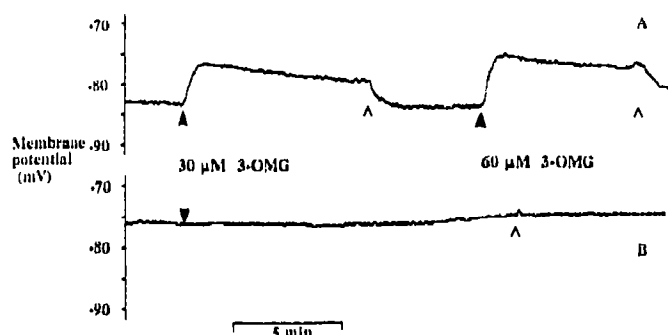


Fig. 4. Membrane potential changes elicited by the addition of 3-OMG to the bathing solution for oocytes injected with (A) STP1 mRNA or (B) water. The membrane potential measurements were made 4 days after injection of the oocytes. In (A) 3-OMG was applied at 30 and 60 μ M, giving initial depolarizations of 6 and 8 mV, respectively. Arrows indicate when the hexose was added (closed) or removed (open).

concentration. This demonstrates that the expression of the STP1 mRNA in oocytes led to the functional expression of an active hexose transport system, not simply a facilitator of 3-OMG diffusion.

Further evidence for the functional expression of the H^+ /hexose co-transporter was provided by the pH dependence of 3-OMG uptake (Fig. 3). While uptake of 3-OMG by water-injected oocytes was independent of pH between 5 and 8, the maximum rate of 3-OMG uptake by oocytes injected with STP1 mRNA was achieved at pH 5. This result is consistent with STP1 being a H^+ co-transporter because this pH, of those tested, provides the largest proton gradient across the oocyte plasma membrane. Interestingly, uptake of radiolabelled 3-OMG via STP1 occurs when there is no gradient of pH (at pH 8, Fig. 3). This result suggests that in the absence of a proton gradient STP1 may be able to use the electrical gradient to drive 3-OMG uptake, or that the protein can also function to facilitate diffusion of hexose.

The symport of hexose and protons across the plasma membrane is expected to give a net movement of the equivalent one unit of positive charge into the oocyte, resulting in a depolarization of the membrane potential. A microelectrode was therefore used to monitor the oocyte membrane potential when oocytes injected with water or STP1 mRNA were superfused with 3-OMG. While water-injected oocytes showed no change in membrane potential on addition of 30 μ M 3-OMG (trace B in Fig. 4), the mRNA-injected oocytes showed a strong depolarization which was reversed on removal of the sugar (trace A in Fig. 4). Similar results were obtained in replicate experiments although the extent of the depolarization varied between individual mRNA-injected oocytes. The size of the depolarization was dependent on the concentration of 3-OMG and was larger with 60 than 30 μ M 3-OMG (trace A in Fig. 4). The endogenous glucose transporter of non-injected oocytes

requires the addition of 10 mM glucose to give a 1–5 mV depolarization [16]. These results provide additional evidence that oocytes injected with STP1 mRNA are expressing a H^+ /hexose co-transporter.

The functional expression of glucose transporters from several animal tissues has been reported to increase the osmotic permeability of oocyte membranes to water [12]. However, when mRNA-injected oocytes that had previously shown a 3-OMG-elicited depolarization were transferred to hypotonic saline, we found no significant difference between their rates of osmotic swelling and those of water-injected oocytes (data not shown).

In conclusion, the finding that a plant membrane protein can be synthesized successfully and targeted to the oocyte plasma membrane to yield a functional transporter clearly demonstrates for the first time the applicability of *Xenopus* oocytes as a heterologous expression system for the identification and characterization of plant transporters and as a potential tool for cloning their genes.

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