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EXPRESSION OF MEMBRANE TRANSPORTERS IN CANE TOAD *BUFO MARINUS* OOCYTES

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Summary

Membrane transport proteins (transporters and ion channels) have been extensively expressed in amphibian oocytes. The aims of this study were to determine whether oocytes from the cane toad *Bufo marinus* could be used as an alternative expression system to the broadly used *Xenopus laevis* oocytes. mRNAs encoding plasma membrane transporters NaSi-1 and sat-1 (sulphate transporters), NaDC-1 (dicarboxylate transporter), SGLT-1 (Na^+/glucose cotransporter) and rBAT and 4F2 hc (amino acid transporters) were injected into *B. marinus* oocytes. All led to significant induction of their respective transport activities. Uptake rates were comparable with those in *X. laevis* oocytes, with the exception of rBAT, which was able to induce amino acid uptake only in *X. laevis* oocytes, suggesting that rBAT may require an endogenous *X. laevis* oocyte protein that is absent from *B. marinus* oocytes. Transport kinetics were determined for the NaSi-1 cotransporter in *B. marinus* oocytes, with identical results to those obtained in *X. laevis* oocytes. NaSi-1 specificity for the Na^+ cation was determined, and the anions selenate, molybdate, tungstate, oxalate and thiosulphate could all inhibit NaSi-1-induced sulphate transport. This study demonstrates that cane toad oocytes can be used successfully to express plasma membrane proteins, making this a viable heterologous system for the expression of proteins.

Key words: protein expression, *Bufo marinus*, *Xenopus laevis*, oocyte, membrane transport, NaSi-1 cotransporter, sat-1 transporter, NaDC-1 cotransporter, SGLT-1 cotransporter, amino acid transport.

Introduction

Frog oocytes have been used widely for the expression of foreign proteins (for reviews, see Colman, 1984; Sigel, 1990). Gurdon et al. (1971) demonstrated that oocytes from the South African clawed toad *Xenopus laevis* were able to synthesise haemoglobin when injected with rabbit haemoglobin mRNA. Since then, this system has proved to be an extremely useful and sensitive method for translating mRNAs from eukaryotes (plants, animals, yeasts) and prokaryotes (bacteria, viruses) (Colman, 1984; Sigel, 1990). This oocyte expression system has been used extensively for expression cloning (Hediger et al., 1987; Bertran et al., 1992b, 1993; Markovich et al., 1993a,b; Bissig et al., 1994; Pajor, 1995) of novel proteins, as well as for studying modes of regulation and structure/function relationships of transporters, ion channels and receptors (Colman, 1984; Dascal, 1987; Sigel, 1990).

The cane toad *Bufo marinus*, indigenous to Central and South America, is a widespread amphibian species that has shown considerable tolerance for reproduction and survival in all regions of the globe. Cane toads were introduced into Australia in June 1935 to combat the sugar cane beetles that were destroying sugar cane in Queensland. However, since then, they have become a biological pest because of their ability to adapt and multiply at an alarming rate (each female toad being able to produce 35 000 oocytes within a single spawn), without reducing the sugar cane beetle numbers. Fully grown oocytes of *B. marinus* are very similar in size to *X. laevis* oocytes and have also been shown to translate rabbit haemoglobin mRNA (May and Glenn, 1974; Glenn and May, 1975). However, unlike with the widely used *X. laevis* oocyte expression system (Sigel, 1990), there have been no subsequent studies that have used *B. marinus* oocytes as a protein translation system for the expression of heterologous proteins of foreign sources.

Because of the widespread abundance and easy accessibility to cane toads, it was our aim to determine whether oocytes of *B. marinus* could be used for the expression of foreign proteins. In this study, we demonstrate that *B. marinus* oocytes can efficiently translate foreign mRNA (encoding several membrane transporters) with protein expression properties identical to those of the well-characterised *X. laevis* oocytes, thereby providing a useful and viable alternative expression system, which could be used for studying membrane proteins of all origins.

Materials and methods

Oocytes and injections

Female *Xenopus laevis* toads were obtained from African

Xenopus Facility C.C., Noordhoek, South Africa. *Bufo marinus* toads (females) were caught locally and maintained in the departmental Animal House. Small clumps of oocytes (total approximately 500–1500 oocytes) were treated for 60–90 min with collagenase Type 4 (2 mg ml^{-1}; Worthington Biochemical Corporation, New Jersey, USA; for *X. laevis* oocytes) or collagenase D (2 mg ml^{-1}; Boehringer Mannheim; for *B. marinus* oocytes) in calcium-free ORII solution (82.5 mmol l^{-1} NaCl, 2 mmol l^{-1} KCl, 1 mmol l^{-1} $MgCl_2$, 10 mmol l^{-1} Hepes/Tris, pH 7.5). Oocytes were then washed thoroughly five times with ORII solution followed by five washes with modified Barth's solution [MBS: 88 mmol l^{-1} NaCl, 1 mmol l^{-1} KCl, 0.82 mmol l^{-1} $MgSO_4$, 0.4 mmol l^{-1} $CaCl_2$, 0.33 mmol l^{-1} $Ca(NO_3)_2$, 2.4 mmol l^{-1} $NaHCO_3$, 10 mmol l^{-1} Hepes/Tris pH 7.4, gentamicin sulphate 20 mg l^{-1}]. The oocytes were sorted for morphologically intact, healthy-looking stage V–VI *X. laevis* oocytes or *B. marinus* oocytes of equal size. They were incubated in MBS at 17 °C and were injected with 50 nl of water (control), 1–3 ng of cRNA per oocyte or 35 ng of mRNA per oocyte using a Nanoject automatic oocyte injector (Drummond Scientific Co., Broomall, PA, USA). Oocytes were then kept at 17 °C in MBS for 1–4 days, with daily changes of MBS.

In vitro *transcription and poly(A^+) RNA isolation*

Rat NaSi-1 (Markovich et al., 1993a), rat sat-1 (Bissig et al., 1994; Markovich et al., 1994), rabbit NaDC-1 (Pajor, 1995), rabbit SGLT-1 (Hediger et al., 1987), human 4F2 hc (Teixeira et al., 1987; Bertran et al., 1992a) and human rBAT (Bertran et al., 1993) cRNAs were synthesised *in vitro* as described previously (Markovich et al., 1993a,b, 1994). Briefly, plasmids (1–2 µg) linearised by restriction enzyme digestion at the 3′ ends of the cloned cDNAs were subjected to the following transcription mixture: transcription buffer 1× (40 mmol l^{-1} Tris/HCl, pH 7.9, 2 mmol l^{-1} spermidine and 6 mmol l^{-1} $MgCl_2$), 0.5 mmol l^{-1} rATP, 0.5 mmol l^{-1} rCTP, 0.5 mmol l^{-1} rUTP, 0.5 mmol l^{-1} m7G(5′)ppp(5′)G, 0.1 mmol l^{-1} rGTP, 10 mmol l^{-1} dithiothreitol, RNAase inhibitor (50 units) and appropriate RNA polymerase (50 units). The reaction mixture was incubated at 37 °C for 2 h, then RNAase inhibitor (50 units) and DNAase I (RNAase-free; 10 units) were added to the samples, which were incubated for a further 15 min at 37 °C. cRNA was then extracted twice with phenol:chloroform:isoamyl alcohol (25:24:1) and precipitated with 1 volume of ammonium acetate (7.5 mol l^{-1}) and 2.5 volumes of ethanol. cRNA was resuspended in 15 µl of water and used directly for injection. Total RNA from *X. laevis* oocytes was isolated by extraction with acid guanidinium thiocyanate/phenol/chloroform and then purified to poly(A^+) RNA (mRNA) using oligo (dT)-cellulose, as described previously (Markovich et al., 1993a,b, 1994).

Oocyte uptakes

Uptakes were performed as described previously (Markovich et al., 1993a,b, 1994). In brief, oocytes (10 oocytes per individual data point) were first washed at room temperature (22–25 °C) for 1–2 min in solution A (100 mmol l^{-1} choline chloride, 2 mmol l^{-1} KCl, 1 mmol l^{-1} $CaCl_2$, 1 mmol l^{-1} $MgCl_2$, 10 mmol l^{-1} Hepes/Tris, pH 7.5). This solution was then replaced by 100 µl of solution B (100 mmol l^{-1} NaCl, 2 mmol l^{-1} KCl, 1 mmol l^{-1} $CaCl_2$, 1 mmol l^{-1} $MgCl_2$, 10 mmol l^{-1} Hepes/Tris, pH 7.5) supplemented with the desired concentration of cold substrate (K_2SO_4, succinate, D-glucose, L-arginine or L-leucine; see figure legends for details) and labelled substrates $Na_2{}^{35}SO_4$, [^{14}C]succinate, D-[^{14}C]glucose, L-[^{3}H]arginine or L-[^{3}H]leucine (New England Nuclear Radiochemicals) (370–740 kBq ml^{-1}). After incubation at room temperature, the uptake solution was removed and the oocytes were washed three times with 3 ml of ice-cold stop solution (solution A). Each single oocyte was then placed into a scintillation vial, dissolved in 250 µl of 1 % SDS, followed by the addition of 2 ml of scintillation fluid (Emulsifier Safe, Canberra Packard) and counted (2 min per oocyte) using liquid scintillation spectrometry.

Data presentation and statistics

Values are given as means ± S.E.M. for 7–10 oocytes per condition and are representative of three similar experiments. Error bars not visible on graphs are smaller than the symbol used for that point. Statistical significance was tested using the unpaired Student's *t*-test, with $P<0.05$ considered significant. For the transport kinetic studies, the Michaelis–Menten and generalised Hill equations were used to calculate the Michaelis constant (K_m) and maximal rate (V_{max}) using non-linear regression.

Results

Initially, cDNAs encoding the following membrane proteins were transcribed *in vitro*: the rat renal Na^+/sulphate cotransporter NaSi-1 (Markovich et al., 1993a), the rat liver sulphate/anion transporter sat-1 (Bissig et al., 1994; Markovich et al., 1994), the rabbit renal Na^+/dicarboxylate cotransporter NaDC-1 (Pajor, 1995), the rabbit intestinal Na^+/glucose cotransporter SGLT-1 (Hediger et al., 1987) and the human amino acid transporters rBAT (Bertran et al., 1993) and 4F2 hc (Teixeira et al., 1987). The cRNAs were injected into the cytoplasm of both *X. laevis* and *B. marinus* oocytes independently and were left for several days to allow the proteins to be translated, modified, sorted and trafficked to the plasma membrane. We then performed radiotracer uptakes as a measure of protein (expressed) activities.

Fully grown *B. marinus* oocytes (1.0–1.5 mm in diameter, approximate volume 1 µl), similar in size to *X. laevis* stage VI oocytes were selected for injection. It is notable that, unlike the distinct hemispherical coloration of *X. laevis* oocytes (a light vegetal pole and a darker animal pole), *B. marinus* oocytes (of all stages) showed no distinctive colour difference, but instead were a uniform jet black (opaque) colour (although some batches did appear to have a very small lighter vegetal pole; data not shown).

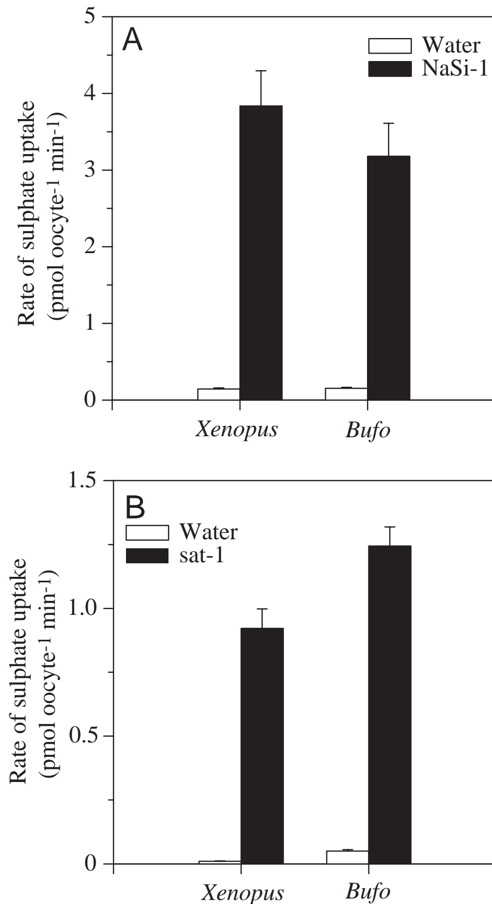

Fig. 1. Expression of NaSi-1 (A) and sat-1 (B) in *Xenopus laevis* and *Bufo marinus* oocytes. Oocytes were injected with water, NaSi-1 cRNA (1 ng per oocyte) or sat-1 cRNA (1 ng per oocyte). $^{35}SO_4^{2-}$ uptakes were measured on day 3 post-injection, using 0.1 mmol l^{-1} K_2SO_4 at room temperature for 30 min. Values are means + S.E.M. (*N*=7–10).

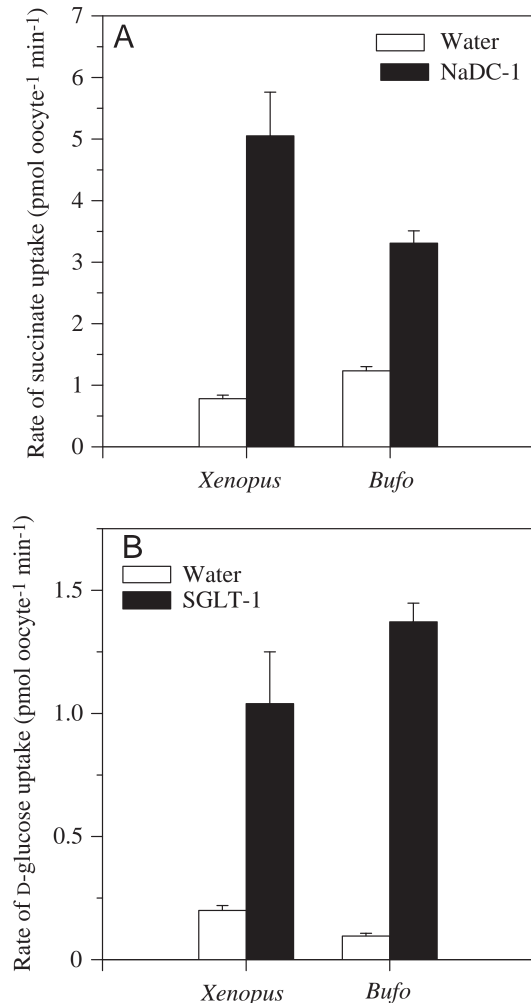

Fig. 2. Expression of NaDC-1 (A) and SGLT-1 (B) in *Xenopus laevis* and *Bufo marinus* oocytes. Oocytes were injected with water, NaDC-1 cRNA (2 ng per oocyte) or SGLT-1 cRNA (2 ng per oocyte). [^{14}C]succinate and D-[^{14}C]glucose uptakes were measured on day 3 post-injection, using 0.1 mmol l^{-1} succinate and 0.1 D-glucose, respectively, at room temperature for 10 min. Values are means + S.E.M. (*N*=7–10).

Oocytes of both *X. laevis* and *B. marinus* showed very similar uptake activities for all the above transport proteins (Figs 1–3), except for rBAT activity (L-leucine uptake) which was expressed only in *X. laevis* oocytes (Fig. 3B). It has been suggested previously that rBAT does not encode a true transporter, but instead an amino acid activator that induces an endogenous amino acid transporter in *X. laevis* oocytes (Bertran et al., 1992b; Taylor et al., 1996; Van Winkle, 1993). To determine whether we could induce the expression of this 'putative' *X. laevis* endogenous protein in *B. marinus* oocytes, we purified mRNA from *X. laevis* oocytes and co-injected it with rBAT cRNA into *B. marinus* oocytes (Fig. 3C). No significant stimulation of L-leucine transport above control rates (water-injected oocytes) was observed in response to either co-injection of *X. laevis* oocyte mRNA with rBAT cRNA or the injection of *X. laevis* oocyte mRNA alone into *B. marinus* oocytes (Fig. 3C). Endogenous oocyte transport activities (water-injected oocytes) for sulphate (Na^+-dependent and Na^+-independent), Na^+/succinate, Na^+/D-glucose and L-leucine uptakes were similar in both *X. laevis* and *B. marinus* oocytes (Figs 1, 2, 3B), with the exception of endogenous L-arginine uptake, which was approximately 2.5 times faster in *B. marinus* oocytes (0.96±0.28 pmol oocyte^{-1} min^{-1}) than in *X. laevis* oocytes (0.36±0.045 pmol oocyte^{-1} min^{-1}; Fig. 3A).

To characterise further protein expression in *B. marinus* oocytes, we performed time-dependence (length of uptake) and time-course (day of uptake) experiments with one of the above transporters, the NaSi-1 cotransporter (Markovich et al., 1993a). *B. marinus* oocytes were injected with NaSi-1 cRNA, and Na^+/sulphate uptake was measured for various times (5–120 min; Fig. 4A). The NaSi-1-induced Na^+/sulphate uptake rate increased linearly up to 120 min. Water-injected oocytes showed insignificant Na^+/sulphate uptake, which remained low up to 120 min (Fig. 4A). Similar results were observed for *X. laevis* oocytes (data not shown). Next, *B. marinus* oocytes were injected with NaSi-1 cRNA, and

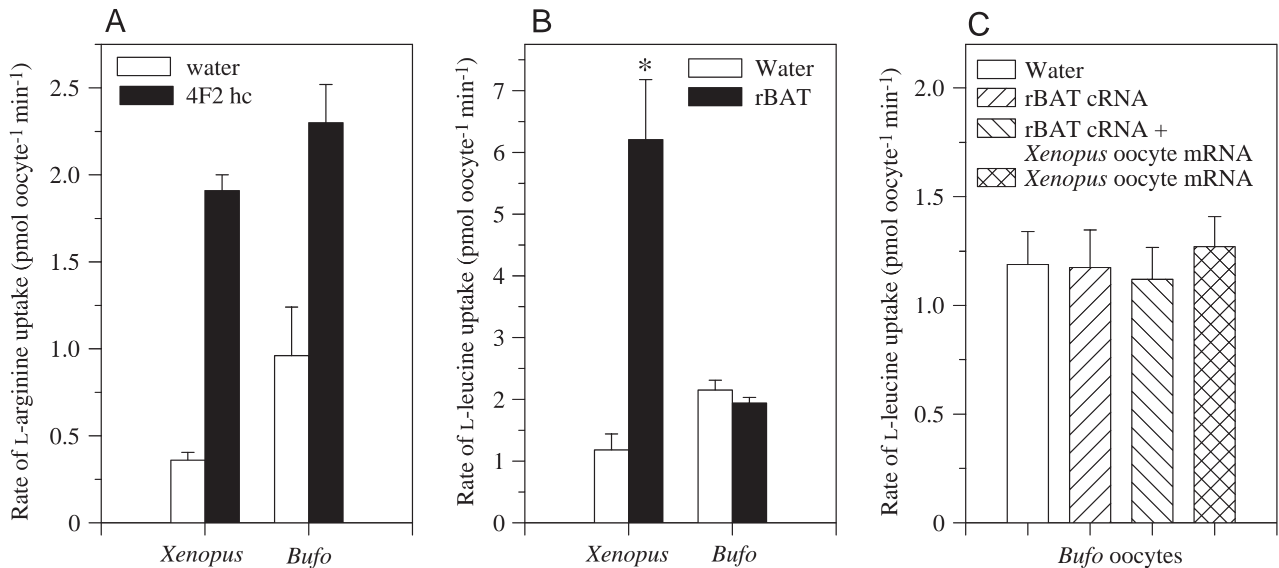

Fig. 3. Expression of 4F2 hc (A) and rBAT (B) in *Xenopus laevis* and *Bufo marinus* oocytes. Oocytes were injected with water, 4F2 hc cRNA (2 ng per oocyte) or rBAT cRNA (2 ng per oocyte). L-[^{3}H]arginine and L-[^{3}H]leucine uptakes were measured on day 3 post-injection, using 0.1 mmol l^{-1} L-arginine and 0.1 mmol l^{-1} L-leucine, respectively, at room temperature for 30 min. (C) Effect of co-injection of rBAT cRNA and *X. laevis* oocyte mRNA into *B. marinus* oocytes. *X. laevis* oocyte mRNA (35 ng per oocyte) was either injected alone or co-injected with human rBAT cRNA (2 ng per oocyte) into *B. marinus* oocytes. L-[^{3}H]leucine uptake was measured on day 2 post-injection, using 0.1 mmol l^{-1} L-leucine, at room temperature for 30 min. Values are means + S.E.M. (N=7–10). An asterisk denotes a statistically significant difference (P<0.05) between *B. marinus* and *X. laevis* rBAT-injected oocytes.

Na^+/sulphate uptake was measured on days 1–4 post-injection (Fig. 4B). NaSi-1-induced Na^+/sulphate uptake rate increased up to day 3 and remained stable thereafter (Fig. 4B). Water-injected oocytes showed insignificant rate of Na^+/sulphate uptake, which remained minimal up to day 4 (Fig. 4B). Similar results were obtained using *X. laevis* oocytes (data not shown).

Furthermore, to determine whether *B. marinus* oocytes could be used for evaluating the kinetic variables (substrate affinities and transport capacity) of membrane proteins, we injected NaSi-1 cRNA into *B. marinus* oocytes and determined its maximal capacity (V_{max}) and substrate affinity (K_m) for sulphate (Fig. 5A) and Na^+ (Fig. 5B). NaSi-1-induced transport in *B. marinus* oocytes showed an exponentially saturable sulphate interaction, representative of Michaelis–Menten kinetics, with the calculated

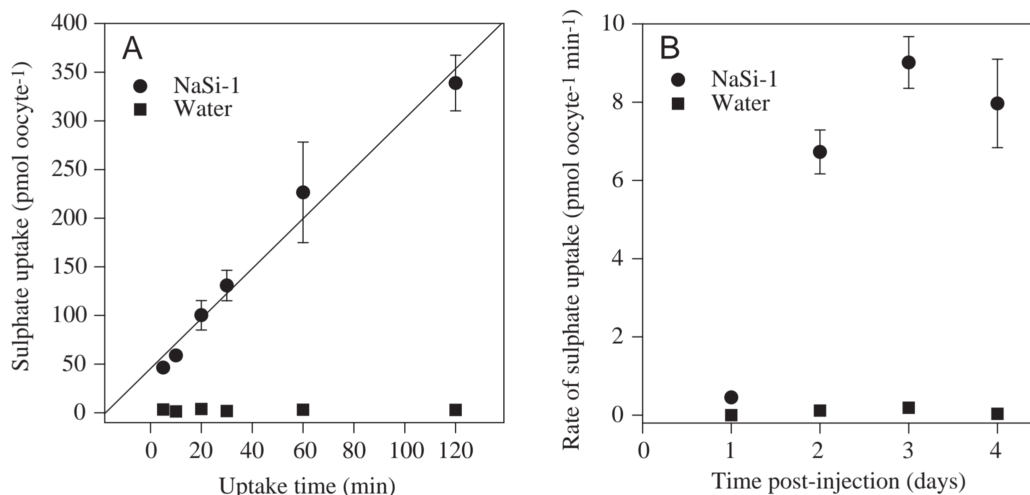

Fig. 4. (A) Time-dependence of NaSi-1-induced sulphate transport in *Bufo marinus* oocytes. Oocytes were injected with water (■) or NaSi-1 cRNA (1 ng per oocyte) (●). $^{35}SO_4^{2-}$ uptake was measured using 0.1 mmol l^{-1} K_2SO_4 in the presence of Na^+ (100 mmol l^{-1}) on day 2 post-injection at room temperature for various times (5–120 min). Values are means ± S.E.M. (N=7–10). The equation for the regression line is y=2.57x+45.4, r^2=0.979, P=0.05. (B) Time course of NaSi-1-induced sulphate transport in *B. marinus* oocytes. Oocytes were injected with water (■) or NaSi-1 cRNA (1 ng per oocyte) (●). $^{35}SO_4^{2-}$ uptakes (30 min at room temperature) were measured using 0.1 mmol l^{-1} K_2SO_4 on various days (1–4) post-injection. Values are means ± S.E.M. (N=7–10).

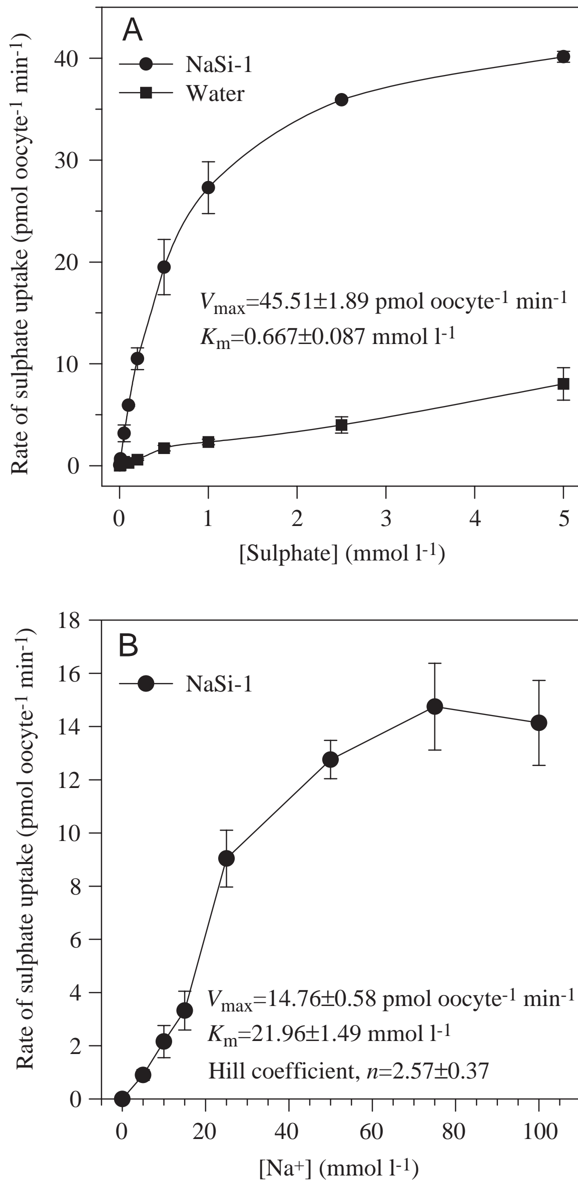

Fig. 5. Effects of sulphate (A) and Na^+ (B) concentration on NaSi-1-induced transport in *Bufo marinus* oocytes. Oocytes were injected with water (■) or NaSi-1 cRNA (1 ng per oocyte) (●). $^{35}SO_4^{2-}$ uptakes were measured on day 2 post-injection at room temperature for 30 min in the presence of various sulphate (0.001–5 mmol l^{-1} K_2SO_4) (A) or Na^+ (0–100 mmol l^{-1} NaCl (B) concentrations. The data were fitted to Michaelis–Menten (A) or generalised Hill (B) equations using non-linear regression. Calculated K_m, V_{max} and n (Hill coefficient) values are presented. Values are means ± S.E.M. (N=7–10).

variables being: V_{max}=45.5±1.9 pmol oocyte^{-1} min^{-1} and K_m=0.67±0.09 µmol l^{-1} for sulphate (Fig. 5A). NaSi-1-induced transport showed a sigmoidal relationship with [Na^+], and when the data were fitted to a generalised Hill equation, the following variables were calculated: V_{max}=14.8±0.6 pmol oocyte^{-1} min^{-1}, K_m=22.0±1.5 mmol l^{-1} for Na^+ and the Hill coefficient n=2.6±0.4. These values are in very close agreement with values

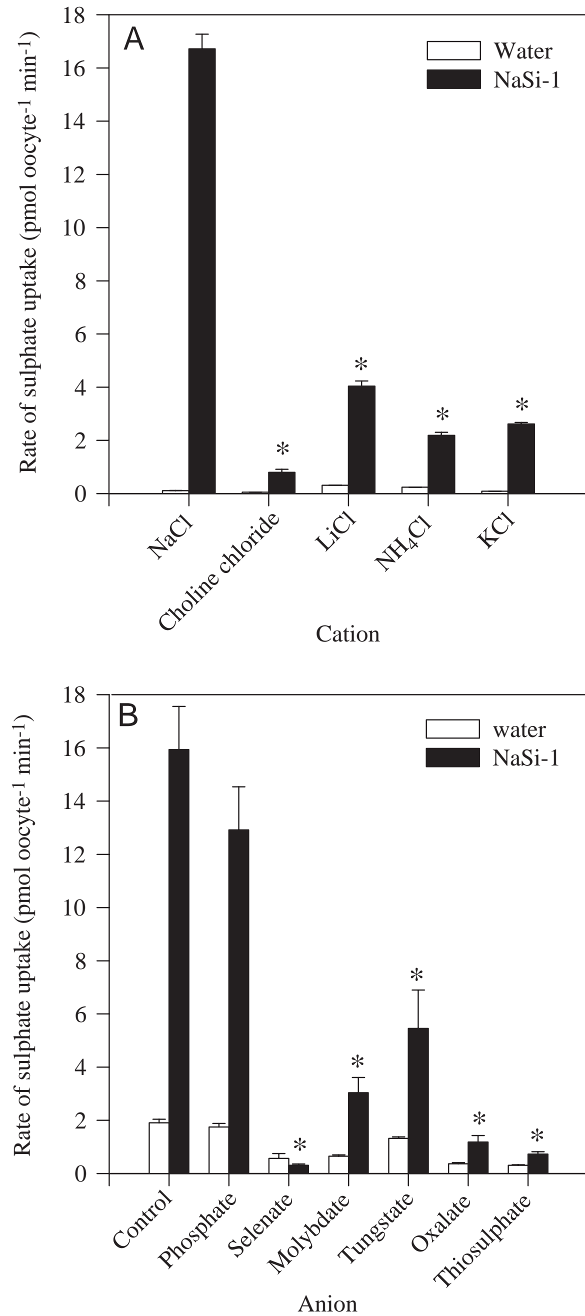

Fig. 6. Cation (A) and anion (B) specificity of NaSi-1-induced transport in *Bufo marinus* oocytes. Oocytes were injected with water or NaSi-1 cRNA (1 ng per oocyte). $^{35}SO_4^{2-}$ uptake was measured on day 3 post-injection using 0.1 mmol l^{-1} K_2SO_4 at room temperature for 30 min. Cation specificity (A) was measured using media containing NaCl, choline chloride, LiCl, NH_4Cl or KCl (each at 100 mmol l^{-1}). An asterisk denotes a statistically significant difference (P<0.05) compared with the NaCl condition. Anion specificity (B) was measured by the addition of phosphate, selenate, molybdate, tungstate, oxalate or thiosulphate (each at 5 mmol l^{-1}) to the uptake medium. Values are means + S.E.M. (N=7–10). An asterisk denotes a statistically significant difference (P<0.05) compared with the control.

obtained in *X. laevis* oocytes (Markovich et al., 1993a; Busch et al., 1994).

Finally, since no study to date has performed a detailed characterisation of the ion specificity of the NaSi-1 cotransporter, we aimed to characterise NaSi-1 anion and cation transport specificity in oocytes (Fig. 6). NaSi-1-induced sulphate transport in *B. marinus* oocytes was maximal in the presence of Na^+, suggesting a strong preference for Na^+, whereas the other cations tested (choline, Li^+, ammonium, K^+) significantly reduced (greater than fourfold) sulphate transport (compared with the Na^+-containing medium; Fig. 6A). NaSi-1-induced Na^+/sulphate cotransport in *B. marinus* oocytes was significantly inhibited by selenate, molybdate, tungstate, oxalate and thiosulphate, but not by phosphate (each at 5 mmol l^{-1}; Fig. 6B). Identical results were observed for the cation and anion specificities of NaSi-1 transport in *X. laevis* oocytes (data not shown).

Discussion

In the present study, our aim was to determine whether cane toad *Bufo marinus* oocytes could be used as a heterologous protein expression system. Our data suggest that *B. marinus* oocytes translate and express (plasma membrane) proteins equally as well as the widely used *X. laevis* oocytes. Several membrane transport proteins, NaSi-1 (Markovich et al., 1993a), sat-1 (Bissig et al., 1994; Markovich et al., 1994), NaDC-1 (Pajor, 1995), SGLT-1 (Hediger et al., 1987) and rBAT (Bertran et al., 1993), encoded by different stuctural entities and having individual substrate specificities, can indeed be expressed in *B. marinus* oocytes at levels comparable with those in *X. laevis* oocytes (Figs 1–3).

The exception is rBAT, which could not induce any amino acid uptake activity in *B. marinus* oocytes, whereas it was clearly expressed in *X. laevis* oocytes (Fig. 3B; Bertran et al., 1992a,b, 1993). The discrepancy could be because rBAT may not be a transporter itself, but is activating an endogenous *X. laevis* transporter that is absent from *B. marinus* oocytes, as we (Bertran et al., 1992b) and others (Van Winkle et al., 1993; Taylor et al., 1996) have previously postulated. Co-injection of *X. laevis* oocyte mRNA with rBAT cRNA into *B. marinus* oocytes did not further stimulate L-leucine (Fig. 3C) or L-arginine (data not shown) transport above levels in control oocytes, suggesting that co-expression of *X. laevis* proteins with rBAT in *B. marinus* oocytes was not sufficient for expression of rBAT-activated amino acid transport.

The fact that rBAT could not be expressed in *B. marinus* oocytes cannot verify or disprove that rBAT is an ‘activator of’ or ‘a protein-encoding’ amino acid transporter, but implies that *B. marinus* oocytes may lack the ability to express rBAT protein and/or lack the endogenous protein that may be activated by rBAT in *X. laevis* oocytes. Alternatively, rBAT may require the presence of another protein (in the oocytes) for proper function. rBAT and 4F2 hc encode structurally related glycoproteins with 52 % sequence similarity, which interact covalently with so-called ‘light chains’ (Bertran et al., 1992a). Recent results have shown that amino acid transport by these two proteins is dependent on the co-expression of their respective ‘light chains’ in *X. laevis* oocytes (Estevez et al., 1998; Palacin et al., 1998). This provides further evidence that rBAT requires an endogenously expressed (light chain) protein in *X. laevis* oocytes for proper transport function. On the basis of these observations, we speculate that *B. marinus* oocytes do not express this rBAT ‘light chain’ and therefore that no amino acid transport is induced in *B. marinus* oocytes, which would make this system ideal for studying the as yet unidentified light chain protein.

Another explanation of why rBAT activity cannot be measured in *B. marinus* oocytes could be that the rBAT mRNA contains consensus sequences, not present in the other transporters tested, rendering it more sensitive to endogenous RNAase H activity in *B. marinus* oocytes, which may be less active in *X. laevis* oocytes. In fact, the 3′ untranslated region of rBAT mRNA contains numerous AT(U)-rich motifs, not present in the other transporters tested, making it more susceptible to RNA degradation (Markovich et al., 1993b). Clearly, further work is needed to resolve these issues.

Previous electrophysiological studies have examined the electrical properties of *B. marinus* oocytes (Dascal, 1987; Iwao et al., 1981). The mean resting membrane potential of *B. marinus* oocytes (–50 mV: Iwao et al., 1981) was found to be identical to that of *X. laevis* oocytes (–50 mV: Dascal, 1987); however, in both species, it varied depending on the method used for defolliculation. Membrane resistance (R_m) in *B. marinus* oocytes were reported to be 10–40 $K\Omega\,cm^{-2}$, with a membrane capacitance of 6–11 $\mu F\,cm^{-2}$, whereas values of R_m as high as 700 $K\Omega\,cm^{-2}$ have been measured in *X. laevis* oocytes, with a comparable membrane capacitance of 4–7 $\mu F\,cm^{-2}$ (Iwao et al., 1981; Dascal, 1987). Intracellular ion concentrations were found to be similar in *B. marinus* and *X. laevis* oocytes (Dascal, 1987). These similarities in electrical, membrane and ionic properties suggest that *B. marinus* oocytes could be used for electrophysiological studies of transporters and ion channels. Previous comparative studies with *X. laevis* oocytes have shown that amino acid transport, urea transport and Cl^- channels are expressed in oocytes from the newt *Cynops pyrrhogaster* (Aoshima et al., 1988) and the frogs *Rana esculenta* (Martial et al., 1991) and *Rana perezi* (Ivorra and Morales, 1997).

Our radiotracer uptake studies demonstrate that *B. marinus* oocytes possess similar functional expression properties to those of *X. laevis* oocytes. We present the following lines of evidence to confirm this: (i) the levels of induction of transport in *B. marinus* oocytes by several transporters (NaSi-1, sat-1, NaDC-1, SGLT-1, 4F2 hc) are similar in magnitude to those in *X. laevis* oocytes; (ii) the levels of endogenous uptake of several substrates (sulphate, succinate, D-glucose and L-leucine) are comparable; (iii) the kinetics of the uptake are analogous; and (iv) the kinetic variables (V_{max} and K_m) detemined are similar in both species. We also observed that, in both *X. laevis* and *B. marinus* oocytes, the NaSi-1 cotransporter shows a strong preference for the Na^+ cation, with insignificant transport when Na^+ is replaced with choline, Li^+, ammonium or K^+. Thiosulphate and the tetra-oxyanions

selenate, molybdate, tungstate and oxalate, but not phosphate, were all able to block significantly the NaSi-1-induced sulphate transport in *X. laevis* and *B. marinus* oocytes, probably by competing for the sulphate-binding site because of their structural (molecular) similarities to the sulphate anion. On the basis of these data, we believe that the *B. marinus* oocytes can be used as a viable alternative to the well-characterised *X. laevis* oocyte expression system.

In conclusion, this is the first study to show that oocytes of the cane toad *B. marinus* can be used successfully for the expression of membrane proteins. We propose that the widespread availability of the *B. marinus* species, especially in Australia, Asia and the Americas, would allow it to be used as a viable alternative and a more readily accessible system for the expression of proteins of all origins.

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References

Aoshima, H., Tomita, K. and Sugio, S. (1988). Expression of amino acid transport systems in *Xenopus* oocytes injected with mRNA from rat small intestine and kidney. *Arch. Biochem. Biophys.* **265**, 73–81.

Bertran, J., Magagnin, S., Werner, A., Markovich, D., Biber, J., Testar, X., Zorzano, A., Kuhn, L. C., Palacin, M. and Murer, H. (1992a). Stimulation of system y(+)-like amino acid transport by the heavy chain of human 4F2 surface antigen in *Xenopus laevis* oocytes. *Proc. Natl. Acad. Sci. USA* **89**, 5606–5610.

Bertran, J., Werner, A., Chillaron, J., Nunes, V., Biber, J., Testar, X., Zorzano, A., Estivill, X., Murer, H. and Palacin, M. (1993). Expression cloning of a human renal cDNA that induces high affinity transport of L-cystine shared with dibasic amino acids in *Xenopus* oocytes. *J. Biol. Chem.* **268**, 14842–14849.

Bertran, J., Werner, A., Moore, M. L., Stange, G., Markovich, D., Biber, J., Testar, X., Zorzano, A., Palacin, M. and Murer, H. (1992b). Expression cloning of a cDNA from rabbit kidney cortex that induces a single transport system for cystine and dibasic and neutral amino acids. *Proc. Natl. Acad. Sci. USA* **89**, 5601–5605.

Bissig, M., Hagenbuch, B., Stieger, B., Koller, T. and Meier, P. J. (1994). Functional expression cloning of the canalicular sulfate transport system of rat hepatocytes. *J. Biol. Chem.* **269**, 3017–3021.

Busch, A. E., Waldegger, S., Herzer, T., Biber, J., Markovich, D., Murer, H. and Lang, F. (1994). Electrogenic cotransport of Na^+ and sulfate in *Xenopus* oocytes expressing the cloned $Na^+SO_4^{2-}$ transport protein NaSi-1. *J. Biol. Chem.* **269**, 12407–12409.

Colman, A. (1984). Translation of eukaryotic messenger RNA in *Xenopus* oocytes. In *Transcription and Translation: A Practical Approach* (ed. B. D. Hames and S. J. Higgins), pp. 271–302. Oxford, UK: IRL Press Ltd.

Dascal, N. (1987). The use of *Xenopus* oocytes for the study of ion channels. *CRC Crit. Rev. Biochem.* **22**, 317–387.

Estevez, R., Camps, M., Rojas, A. M., Testar, X., Deves, R., Hediger, M. A., Zorzano, A. and Palacin, M. (1998). The amino acid transport system y+L/4F2hc is a heteromultimeric complex. *FASEB J.* **12**, 1319–1329.

Glenn, A. R. and May, B. K. (1975). Effect of haemin on endogenous protein synthesis in oocytes of the Queensland cane toad *Bufo marinus*. *Aust. J. Biol. Sci.* **28**, 115–119.

Gurdon, J. B., Lane, C. D., Woodland, H. R. and Mairbaix, G. (1971). Use of frog eggs and oocytes for the study of messenger RNA and its translation in living cells. *Nature* **233**, 177–182.

Hediger, M. A., Coady, M. J., Ikeda, T. S. and Wright, E. M. (1987). Expression cloning and cDNA sequencing of the Na^+/glucose co-transporter. *Nature* **330**, 379–381.

Ivorra, I. and Morales, A. (1997). Membrane currents in immature oocytes of the *Rana perezi* frog. *Pflügers Arch.* **434**, 413–421.

Iwao, Y., Ito, S. and Katagiri, C. (1981). Electrical properties of toad oocytes during maturation and activation. *Dev. Growth Differ*. **23**, 89–100.

Markovich, D., Bissig, M., Sorribas, V., Hagenbuch, B., Meier, P. J. and Murer, H. (1994). Expression of rat renal sulfate transport systems in *Xenopus laevis* oocytes: functional characterization and molecular identification. *J. Biol. Chem.* **269**, 3022–3026.

Markovich, D., Forgo, J., Stange, G., Biber, J. and Murer, H. (1993a). Expression cloning of rat renal Na^+/SO_4^{2-}-cotransport. *Proc. Natl. Acad. Sci. USA* **90**, 8073–8077.

Markovich, D., Stange, G., Bertran, J., Palacin, M., Werner, A., Biber, J. and Murer, H. (1993b). Two mRNA transcripts (rBAT-1 and rBAT-2) are involved in system b0,(+)-related amino acid transport. *J. Biol. Chem.* **268**, 1362–1367.

Martial, S., Ripoche, P. and Ibarra, C. (1991). Functional expression of urea channels in amphibian oocytes injected with frog urinary bladder mRNA. *Biochim. Biophys. Acta* **1090**, 86–90.

May, B. K. and Glenn, A. R. (1974). Translation of rabbit haemoglobin mRNA in oocytes of the Queensland cane toad, *Bufo marinus*. *Aust. J. Biol. Sci.* **27**, 585–589.

Pajor, A. M. (1995). Sequence and functional characterization of a renal sodium/dicarboxylate cotransporter. *J. Biol. Chem.* **270**, 5779–5785.

Palacin, M., Estevez, R. and Zorzano, A. (1998). Cystinuria calls for heteromultimeric amino acid transporters. *Curr. Opin. Cell Biol.* **10**, 455–461.

Sigel, E. (1990). Use of *Xenopus* oocytes for the functional expression of plasma membrane proteins. *J. Membr. Biol.* **117**, 201–221.

Taylor, P. M., Kaur, S., Mackenzie, B. and Peter, G. J. (1996). Amino-acid-dependent modulation of amino acid transport in *Xenopus laevis* oocytes. *J. Exp. Biol.* **199**, 923–931.

Teixeira, S., Di Grandi, S. and Kuhn, L. C. (1987). Primary structure of the human 4F2 antigen heavy chain predicts a transmembrane protein with a cytoplasmic NH_2 terminus. *J. Biol. Chem.* **262**, 9574–9580.

Van Winkle, L. J. (1993). Endogenous amino acid transport systems and expression of mammalian amino acid transport proteins in *Xenopus* oocytes. *Biochim. Biophys. Acta* **1154**, 157–172.