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Transport of Glutamine in *Xenopus laevis* Oocytes: Relationship with Transport of Other Amino Acids

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Summary. We have investigated transport of the amino acid glutamine across the surface membranes of prophase-arrested *Xenopus laevis* oocytes. Glutamine accumulation was linear with time for 30 min; it was stereospecific with a K_m of 0.12 ± 0.02 mM and V_{max} of 0.92 ± 0.17 pmol/oocyte · min for L-glutamine. Transport of L-glutamine was Na^+-dependent, the cation not being replaceable with Li^+, K^+, choline, tris(hydroxymethyl)-aminomethane (Tris), tetramethylammonium (TMA) or N-methyl D-glucamine (NMDG); external Cl^- appeared to be necessary for full activation of Na^+-dependent glutamine transport. Two external Na^+ may be required for the transport of one glutamine molecule. L-glutamine transport (at 50 μM glutamine) was inhibited by the presence of other amino acids: L-alanine, D-alanine, L-leucine, L-asparagine and L-arginine (about 60% inhibition at 1 mM); L-histidine, L-valine and glycine (25 to 40% inhibition at 1 mM); L-serine, L-lysine, L-phenylalanine and L-glutamate (45 to 55% inhibition at 10 mM). N-methylaminoisobutyric acid (MeAIB) had no effect at 10 mM, but 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid (BCH) inhibited Na^+/glutamine transport by about 50% at 10 mM. L-glutamine was a competitive inhibitor of the Na^+-dependent transport of L-alanine, D-alanine and L-arginine; this evidence is consistent with the existence of a single system transporting all four amino acids. Glutamine uptake in oocytes appears to be catalyzed by a transport system distinct from the cotransport Systems A, ASC, N and Gly, although it resembles System $B^{0,+}$.

Key Words *Xenopus* oocyte · amino acid · Na^+-dependent transport · glutamine

Introduction

A potentially powerful approach to gaining information about the molecular structure of transport proteins is via identification of messenger RNA (mRNA) complementary to the encoding DNA, as has been achieved recently for the intestinal Na^+/glucose cotransporter (Hediger *et al.*, 1987). In that study prophase-arrested amphibian oocytes (from the toad *Xenopus*) were used as an expression system to screen mRNAs, taking advantage of the fact that the oocytes efficiently translate exogenous mRNAs and are capable of expressing foreign transport proteins in the oocyte plasma membranes (e.g., Colman, 1984). We have kinetically characterized the related mammalian amino acid transport systems N and N^m, for which the metabolically important amino acid glutamine is the major substrate, in perfused rat liver and muscle, respectively (Hundal, Rennie & Watt, 1987; Taylor & Rennie, 1988). However, the isolation of this, and other, amino acid transporters has proved to be difficult by conventional techniques used for membrane bound proteins (e.g., the red cell anion transporter; for summary *see* Kopito & Lodish, 1985). It is our eventual aim to use *Xenopus* oocytes as an expression vector for glutamine transport proteins encoded by mammalian poly(A)-RNA, and a prerequisite is knowledge of the endogenous mechanisms for glutamine transport in the oocyte membranes.

Amino acid transport in animal oocytes has been reviewed recently (Van Winkle, 1988). It appears that there is no full description at present of the modes of amino acid transport through the outer membranes of *Xenopus* oocytes. In prophase-arrested oocytes transport of glutamine, together with transport of alanine, glycine, glutamate, and tyrosine results in their concentration intracellularly, suggesting the involvement of an energy requiring process, possibly secondary active transport. Accordingly, alanine transport in prophase-arrested oocytes has been shown to be Na^+-dependent with an apparent stoichiometry of two Na^+ ions transported per alanine molecule (Jung, Schwarz & Passow, 1984*b*). Kinetic characteristics (K_m, V_{max}) have been reported for several amino acids, but glutamine is not among them. Na^+-dependent alanine transport in oocytes has a V_{max} value of about 1.2 pmoles/oocyte · min and a K_m value of about 100 μM: the transport K_ms for most other amino acids studied are below 1 mM. It has recently been suggested (Van Winkle, 1988) that *Xenopus* oocyte

membranes contain an unusual Na^+-dependent amino acid transporter of wide specificity, although other authors (Bravo, Salazar & Allende, 1976; Jung et al., 1984*b*) had concluded that transporters similar to those described in mammalian tissues (for review *see* Christensen & Kilberg, 1987), e.g., the Na^+-dependent system A and the Na^+-independent system L, were present.

We report here the results of experiments carried out to characterize glutamine transport in prophase-arrested *Xenopus* oocytes.

Materials and Methods

Chemicals were obtained from Sigma Chemical (Poole, UK), unless otherwise specified; radiochemicals were obtained from Amersham International (Amersham, UK). [^{3}H]-labeled amino acids were obtained at a specific activity of 1.5–6 TBq/mmol and [^{14}C]-labeled amino acids were obtained at a specific activity of 1.5–10 GBq/mmol.

Toads (*Xenopus laevis*) were obtained from Griffin & George (Loughborough, UK) and maintained in fresh water aquaria. The procedures followed for oocyte preparation were as described by Colman (1984). Briefly, pieces of ovary were removed from *Xenopus* (under 0.1% aminoethylbenzoate anaesthesia), rinsed rapidly and incubated for 3–4 hr at 20°C in modified Barth's solution containing 2 mg/ml of collagenase (Boehringer, Lewes, UK). The composition of the modified Barth's solution is (in mM): 88 NaCl, 1 KCl, 2.4 $NaHCO_3$, 0.82 $MgSO_4$, 0.33 $Ca(NO_3)_2$, 0.41 $CaCl_2$, 5.0 HEPES, pH 7.6; penicillin and streptomycin were added at 10 mg/liter each. Prophase-arrested stage-VI oocytes were then collected and incubated in Barth's medium at 20°C. All experiments were performed on healthy oocytes incubated for between 20 and 24 hr, unless otherwise specified. Oocytes were transferred between media using a Pasteur pipette with a wide-bore tip.

Amino acid uptake into oocytes was measured by a radiotracer technique. In this, oocytes were incubated in experimental medium (100 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES/Tris at pH 7.5: Hediger et al., 1987) containing a radioactive amino acid for a fixed time period at 22–25°C. Uptake was terminated by rapidly removing the oocytes from the incubation medium, rinsing them in distilled water (DW) and transferring them individually in 0.2 ml DW to 10 ml capacity plastic scintillation vials. Oocytes burst within 30 min of transfer (presumably due to osmotic swelling) and 0.2 ml of 2% Triton-X 100 was then added to the disrupted cell suspension. 3 ml of scintillant ("Ready-Value," Beckman) was added to each vial and sample radioactivity was assayed by liquid scintillation counting (Beckman LS 1800), with standard quench correction procedures being used to yield disintegrations/minute (dpm). 0.02-ml aliquots of experimental medium were prepared for counting in parallel with oocytes. In certain experiments oocyte protein was isolated from the cell suspension by precipitation with 5 volumes of ice-cold acetone: the protein pellet was redissolved in 0.4 ml of 1% Triton/0.5 M NaOH, transferred to a vial containing 3 ml scintillant and assayed for radioactivity as described above. In preliminary experiments (results not shown) we established that neither our defolliculation procedure (i.e., 4 hr collagenase treatment), nor a prolonged collagenase-incubation procedure designed to ensure complete removal of the follicle cell layer (Mohun et al., 1981), significantly affected the rate of [^{3}H]glutamine uptake in oocytes (removal of the follicle cell layer was confirmed using scanning electron microscopy). These results demonstrated that measured rates of radiotracer uptake reflected amino acid transport into the oocyte, and not into surrounding cell layers, and confirmed the results of previous studies (Bravo et al., 1976; Hallberg & Smith, 1976; Otero et al., 1978).

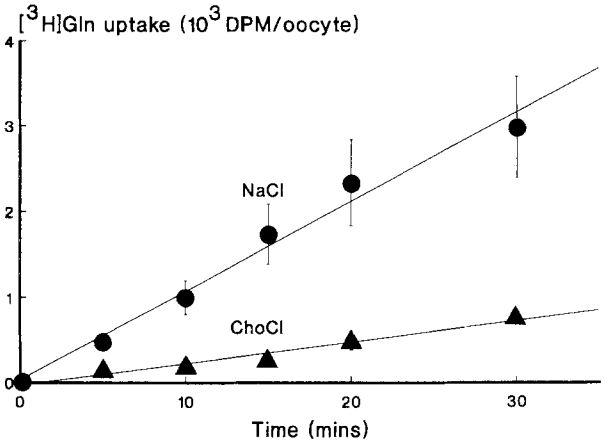

Fig. 1. Time course of [^{3}H]-glutamine uptake in *Xenopus* oocytes (50 μM glutamine (400 dpm/pmol); 100 mM cation chloride). n = 8–15 oocytes at each time point (mean ± SEM.)

The cation dependence of amino acid uptake into oocytes was investigated using chlorides of choline (Cho), Li^+, K^+, tris(hydroxymethyl)aminomethane (Tris), tetramethylammonium (TMA) or N-methyl D-glucamine (NMDG) to substitute for NaCl in the experimental medium. Anion dependence was studied by replacing NaCl with $NaNO_3$, NaSCN or Na-acetate; Cl^- concentration in these media was reduced from 106 to 6 mM.

Amino acid uptake into oocytes is expressed as pmoles/oocyte · min. All results are presented as the mean ± 1 standard error (SEM), and n represents the number of observations (the number of oocytes used in a single experiment varied between 8 and 20). The study was performed using oocytes from 10 different toads. The difference between sample means was tested for significance using Student's t test; differences were considered significant if $P < 0.05$. Lines or curves were fitted to data (by least-squares and iterative methods, respectively) using commercial software (Barlow, 1982) run on an Apple IIe microcomputer.

Results

The rate of L-[^{3}H]glutamine uptake was linear over the first 30 min of incubation (Fig. 1), and it appeared that oocyte glutamine transport included a Na^+-dependent component, since the uptake rate in ChoCl was much lower than that in NaCl. Oocytes continued to accumulate [^{3}H]-glutamine if the incubation time was extended to 4 hr (Table 1), by which time significant amounts of tracer had been incorporated into oocyte proteins. A 20-min incubation period was chosen for convenience in all further ex-

Table 1. Uptake of [^{3}H]glutamine by *Xenopus* oocytes: 50 μM glutamine (400 dpm/pmol)

Condition	[^{3}H]glutamine uptake (dpm)	
	20 min	4 hr
Control	2330 ± 510	19100 ± 3300
Oocyte protein	305 ± 80	5900 ± 1450
Control + 1 mM L-Gln	415 ± 25	—
Control + 1 mM D-Gln	2260 ± 275	—

All values are mean ± 1 SEM; n = 9–11 oocytes from a single toad.

Table 2. Cation dependence of L-glutamine transport in *Xenopus* oocytes: 50 μM L-glutamine, 100 mM cation chloride

Cation	L-glutamine influx (pmol/oocyte · min)
Sodium	0.252 ± 0.022[a]
Choline	0.053 ± 0.013
Lithium	0.077 ± 0.011
Potassium	0.071 ± 0.013
NMDG	0.053 ± 0.009
Tris	0.066 ± 0.014
TMA	0.060 ± 0.008

NMDG, N-methyl D-glucamine; Tris, Tris(hydroxymethyl)-aminomethane; TMA, tetramethylammonium.
n = at least 10 oocytes from a single toad.
[a] value significantly different from all others, $P < 0.001$.

Table 3. Anion dependence of L-glutamine transport in *Xenopus* oocytes: 50 μM L-glutamine, 100 mM Na salt

Anion	L-Glutamine influx (pmol/oocyte · min)
Chloride	0.327 ± 0.063[a]
Nitrate	0.149 ± 0.022
Acetate	0.176 ± 0.011
Thiocyanate	0.178 ±0.034

n = 9 oocytes from a single toad.
[a] Value significantly different from others; $P < 0.05$.

periments; under 15% of accumulated [^{3}H]activity had become protein-bound by this time.

L-[^{3}H]glutamine uptake into oocytes was inhibited by the presence of L-glutamine but not by D-glutamine (both at 1 mM; Table 1), indicating that the uptake mechanism was both saturable and stereospecific.

The replacement cations Cho, Li^+, K^+, TMA, Tris and NMDG could not effectively substitute for Na^+ in stimulating glutamine uptake into oocytes (Table 2). Neither Li^+ nor Cho appeared to have any independent effect on Na^+/glutamine transport at the concentrations used, as glutamine uptakes in media containing 180 mM NaCl, 100 mM NaCl + 80 mM LiCl, or 100 mM NaCl + 80 mM ChoCl did not differ significantly from one another, nor from glutamine uptake at 100 mM NaCl (data not shown). Replacement of Cl^- with nitrate, acetate or thiocyanate significantly inhibited Gln uptake into oocytes (Table 3).

Progressive replacement of NaCl with ChoCl revealed an apparently S-shaped relationship between Na^+-dependent L-glutamine transport (v, i.e., uptake in Na^+ medium minus uptake in ChoCl) and external Na^+ concentration (Fig. 2*a*); we could not obtain a reasonable estimate for v at tested Na^+ concentrations below 20 mM (i.e., 5 and 10 mM), because total glutamine uptake under these conditions was not significantly greater than that in ChoCl medium. An Eadie-Hofstee plot of the data (that is, v *vs.* $v/[Na^+]^n$) produced a good fit to a straight line when $n = 2$ ($r = -0.932$ with 4 degrees of freedom (df); Fig. 2*b*) but not when $n = 1$; a similar result was obtained when Li^+ was used to replace Na^+ (data not shown). It therefore appeared *a priori* that two external Na^+ were required for the transport of a single glutamine molecule.

Using self-inhibition of tracer by native glutamine (0.002, 0.01, 0.05, 0.1, 0.5, 1 and 5 mM glutamine in NaCl medium; 0.05, 0.5, 1 and 5 mM glutamine in ChoCl medium) we demonstrated that it was the Na^+-dependent component of glutamine uptake which was saturable at external glutamine concentrations below 1 mM (Fig. 3). The dependence on glutamine concentration of Na^+-dependent glutamine transport in oocytes from individual toads was computer analyzed by curve fitting to a hyperbola; this analysis gave mean values of 0.12 mM for K_m and 0.92 pmol/oocyte · min for V_{max} (Table 4). A least-squares line fit to linear-transformed data (Hanes plot) confirmed these values. At 5 mM glutamine Na^+-independent glutamine uptake into oocytes predominated (3.9 ± 0.9 and 3.0 ± 0.4 pmol/oocyte · min in NaCl and ChoCl, respectively, N = 5 toads) but a mean value for Na^+-dependent uptake (estimated by difference) was, at ~0.9 pmol/oocyte · min, of similar magnitude to the calculated transport V_{max}.

In order to investigate the specificity of L-glutamine transport, the effects of other amino acids on the rate of uptake of glutamine into oocytes were determined (Fig. 4). L-Alanine, D-alanine, L-leucine, L-asparagine and L-arginine were the most effective inhibitors of glutamine uptake among the natural amino acids tested, but L-lysine, L-histidine, L-valine, L-glutamate, glycine and L-phenylalanine were also inhibitors to some extent. The amino acid analog 2-aminobicyclo[2,2,1]heptane-2-carboxylic

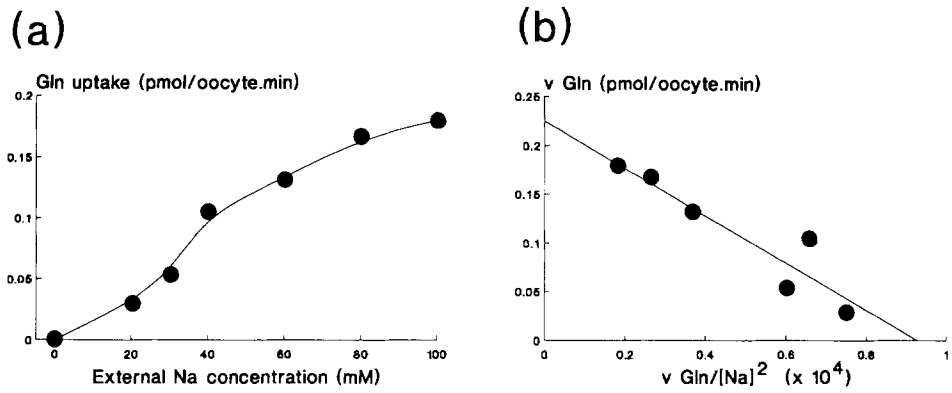

Fig. 2. (*a*) Na^+-dependent L-glutamine transport in *Xenopus* oocytes. Each point is the mean Na^+-stimulated glutamine uptake of 10 oocytes at 50 μM external glutamine (SEMS ~11 ± 2%): NaCl was replaced with ChoCl to maintain 100 mM cation chloride. (*b*) Eadie-Hofstee plot of data in *a*, with *v* glutamine/$[Na^+]^2$ at the *x* axis

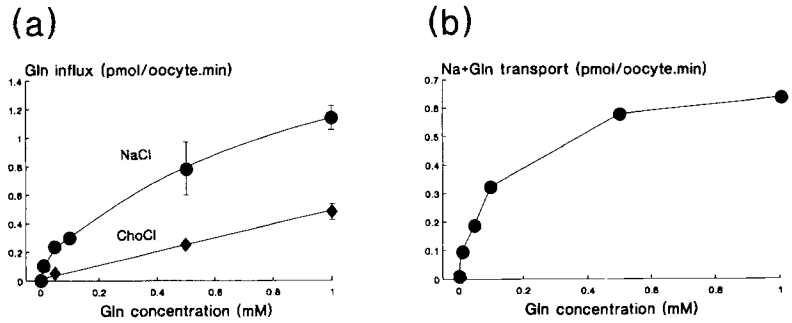

Fig. 3. Influx of L-glutamine in *Xenopus* oocytes as a function of external glutamine concentration. These representative results were obtained using oocytes from a single toad. (*a*) Total glutamine influx. (*b*) Na^+-dependent glutamine transport, calculated at each glutamine concentration as the mean influx in NaCl minus the influx in ChoCl (the latter was estimated from the fitted line shown in *a*)

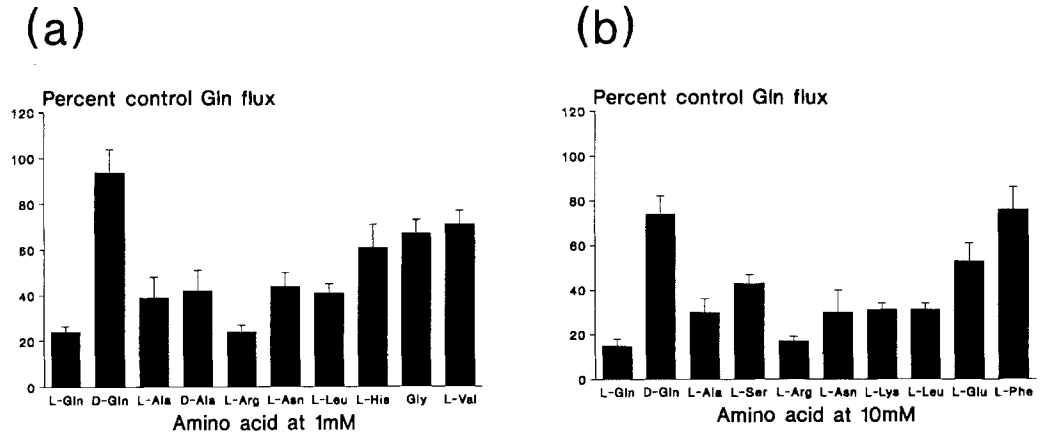

Fig. 4. Inhibition of 50 μM L-glutamine transport in *Xenopus* oocytes by other amino acids at (*a*) 1 mM and (*b*) 10 mM. All values are mean ± SEM; n = 4 to 10 toads

acid (BCH) inhibited the Na^+-dependent component of oocyte glutamine uptake, but another analog, N-methylaminoisobutyric acid (MeAIB; a specific substrate of System A), was without effect on glutamine uptake (Table 5).

Inhibition of Na^+-dependent glutamine transport by the addition of other amino acids to the external medium, or by the replacement of Cl^- with anions such as NO_3^-, does not necessarily reflect a direct effect on the transport mechanism because

Table 4. Kinetic characteristics of Na-dependent L-glutamine (Gln) transport in oocytes from five *Xenopus* toads at 100 mM NaCl

Toad	K_m (mM Gln)	V_{max} (pmol Gln/oocyte · min)
1	0.11	0.71
2	0.10	1.44
3	0.14	0.54
4	0.18	0.72
5	0.06	1.21
Mean ± SEM	0.12 ± 0.02	0.92 ± 0.17

Standard errors for oocytes of individual toads (10 oocytes at each of six glutamine concentrations between 0.002 and 1 mM) were about 7% for V_{max} and 25% for K_m.

Table 5. The effect of synthetic amino acid analogs on 50 μM L-glutamine uptake by *Xenopus* oocytes: n = three toads

Condition	L-glutamine uptake (pmol/oocyte · min)	
	100 mM NaCl	100 mM ChoCl
control	0.279 ± 0.049	0.032 ± 0.011
10 mM BCH	0.145 ± 0.009[a]	0.041 ± 0.013
10 mM MeAIB	0.260 ± 0.040	0.035 ± 0.009

BCH: 2-aminobicyclo[2,2,1]heptane-2-carboxylic acid; MeAIB: 2-(methylamino)isobutyrate.
[a] Value significantly different from control; $P < 0.05$.)

the experimental manipulations could result in the depolarization of the oocyte membrane potential (E_m), and depolarization has been shown to inhibit Na-dependent amino acid transport in oocytes (Jung, Lafaire & Schwarz, 1984*a*). Electrogenic cotransport of Na and alanine depolarizes E_m by ~10 mV (from a resting value of ~−65 mV) if alanine is added to the external medium at a concentration supramaximal for transport (i.e., >1 mM; Jung et al., 1984*b*). Other amino acids undergoing cotransport might be expected to produce depolarizations of about the same magnitude, because the kinetic characteristics for uptake of a wide variety of amino acids by oocytes are of much the same order (*see* Van Winkle (1988) and Tables 4 and 6–8 in the present paper). Replacement of external NaCl with $NaNO_3$ results in a slight hyperpolarization of oocyte E_m (by ~8 mV; M.R. Ward, *personal communication*). Changes in oocyte E_m of the above order (± 10 mV) should not significantly affect the rate of Na+amino acid cotransport (Jung et al., 1984*a*); therefore, it appears likely that most of the effects observed in our experiments are to a large extent attributable to direct inhibition of the Na-dependent glutamine transport mechanism.

The effects of (i) Na^+ replacement, and (ii) 1 mM glutamine and 1 mM arginine, on the initial (20 min) uptakes of radiotracers for a variety of amino acids are summarized in Tables 6 and 7 (preliminary experiments had shown that the rate of uptake of these tracers was linear over 20 min). L-Glutamine, L-alanine, D-alanine and L-arginine were all transported mainly by Na^+-dependent mechanisms and showed strong mutual inhibition of transport, indicating that they may share the same transport mechanism. We performed further experiments on single batches of oocytes to see if the inhibition between L-glutamine, L-alanine and D-alanine (toad 1 oocytes: *see* Table 4), and L-glutamine and L-arginine (toad 5 oocytes) was competitive or noncompetitive. We obtained values for kinetic characteristics of L-alanine, D-alanine and L-arginine uptake in the oocytes in the same manner used previously for glutamine, then we attempted to obtain values for V_{max} and K_m of uptake of these three amino acids from experiments performed in the presence of 50 or 100 μM L-glutamine; we assumed that only Na^+-dependent amino acid uptake was inhibited. The results, summarized in Table 8, indicate that the presence of L-glutamine caused an apparent increase in K_m for transport of all three amino acids but did not significantly affect transport V_{max}. These findings are consistent with the existence of competitive inhibition between the four amino acids investigated. The apparent K_m for transport in the presence of inhibitor (denoted K_{app}) is equivalent to K_m $(1 + [i]/K_i)$, where $[i]$ is the concentration of inhibitor (0.05 or 0.1 mM glutamine in the present experiments) and K_i, the inhibitor constant, should equal the transport K_m of a competitive inhibitor. Values for K_i of L-glutamine calculated from mean K_{app}'s of L- and D-alanine (~0.095 mM) were indeed not significantly different from the K_m for L-glutamine measured in oocytes from toad 1 (0.11 ± 0.035 mM), although it appeared that V_{max} for either stereoisomer of alanine (~1.15 pmol/oocyte · min) was slightly higher than V_{max} for L-glutamine (0.71 ± 0.07 pmol/oocyte · min). Similarly, the K_i for glutamine as an inhibitor of L-arginine uptake (~0.055 mM) was not significantly different from the glutamine transport K_m of toad 5 oocytes (0.06 ± 0.012 mM), but in this case V_{max} for transport of glutamine and arginine were of similar magnitude to one another (~1.2 pmol/oocyte · min).

Glutamine uptake into oocytes was susceptible to changes of pH in the range 5.5 to 8.5; there was an uptake maximum at about pH 7.5 (Fig. 5).

Table 6. The effect of sodium replacement (by choline) on the rate of amino acid (AA) uptake by *Xenopus* oocytes[a]

Amino acid	Concentration (mM)	AA uptake (pmol/oocyte · min)	
		100 mM NaCl	100 mM ChoCl
TOAD 1			
L-Ala	0.05	0.251 ± 0.025	0.073 ± 0.009[b]
	1.0	1.71 ± 0.39	0.67 ± 0.17[b]
D-Ala	0.05	0.138 ± 0.011	0.057 ± 0.010[b]
	1.0	1.24 ± 0.29	0.57 ± 0.07[b]
TOAD 2			
L-Ala	0.05	0.440 ± 0.046	0.096 ± 0.022[b]
L-Arg	0.05	0.397 ± 0.045	0.065 ± 0.015[b]
L-Val	0.05	0.356 ± 0.023	0.089 ± 0.005[b]
L-Phe	0.05	0.545 ± 0.072	0.188 ± 0.078[b]
L-Glu	0.05	0.198 ± 0.066	0.176 ± 0.061

[a] Summary of experiments using oocytes from two toads.
All values are mean ± 1 SEM: n = 10 oocytes.
[b] Values in ChoCl significantly different from values in NaCl; $P < 0.05$.

Table 7. The effects of L-glutamine and L-arginine on amino acid uptake by *Xenopus* oocytes

Amino acid (50 μM)	% inhibition of amino acid uptake	
	1 mM glutamine	1 mM arginine
L-Alanine	46 ± 2	62 ± 9
D-Alanine	46 ± 7	
L-Arginine	74 ± 8	76 ± 5
L-Glutamate	41 ± 8	
L-Phenylalanine	45 ± 3	
L-Valine	43 ± 4	

Mean ± 1 SEM; n = 10 oocytes from a single toad.

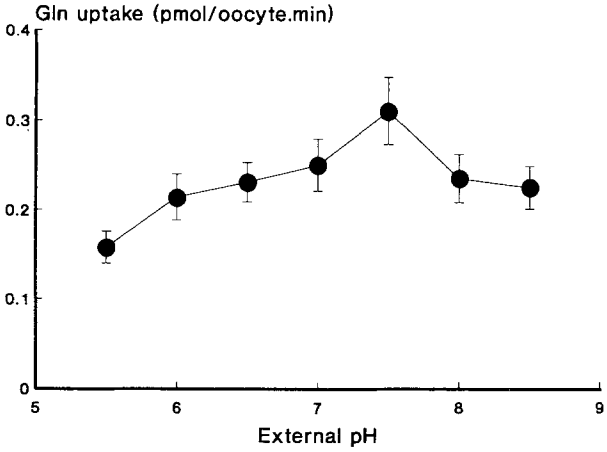

Fig. 5. pH dependence of 50 μM glutamine transport in *Xenopus* oocytes. All values are mean ± 1 SEM; n = 10–20 oocytes

All experiments described above were performed on oocytes held in culture for ~24 hr. Oocytes cultured for longer than ~48 hr progressively lost Na^+/glutamine transport activity and simultaneously increased their activity of Na^+-independent glutamine transport (Fig. 6). A preliminary study of this Na-independent uptake component showed that it was saturable; glutamine, asparagine and glutamate were inhibitory at 1 mM (50–70% inhibition) but leucine, lysine, BCH and phenylalanine were not.

Discussion

At L-glutamine concentrations below about 1 mM the major component of glutamine uptake by prophase-arrested *Xenopus* oocytes cultured in Barth's medium for 24 hr is saturable and Na^+-dependent. L-glutamine, L-alanine, D-alanine and L-arginine appear to be transported in oocytes by the same Na^+-dependent carrier mechanism. Alanine transport by this mechanism appeared to be much less stereospecific than for glutamine, although L-alanine uptake was favored as judged from its lower K_m (0.17 mM *vs.* 0.72 mM for D-alanine). Glutamine transport may not be absolutely specific for the L-isomer because 10 mM D-glutamine did slightly inhibit 50 μM L-glutamine uptake, although 1 mM D-glutamine was ineffective (*see* Fig. 4): 1 mM D-glutamine did not inhibit uptake of 50 μM L- or D-alanine (results not shown). Our values for K_m and V_{max} of Na^+-dependent transport of glutamine, alanine and arginine in prophase-arrested oocytes are of the same

Table 8. The effect of L-glutamine (0.05 or 0.1 mM) on V_{max} and K_m of Na-dependent L-alanine, D-alanine and L-arginine transport in *Xenopus* oocytes

Amino acid	V_{max}	V_{max}^{app}	K_m	K_{app}
	(pmol/oocyte · min)		(mM)	
Toad 1				
L-Ala	1.21 ± 0.23	1.10 ± 0.19	0.17 ± 0.07	0.26 ± 0.06
D-Ala	1.11 ± 0.16	1.20 ± 0.09	0.72 ± 0.19	1.10 ± 0.22
Toad 5				
L-Arg	1.39 ± 0.33	1.12 ± 0.27	0.05 ± 0.02	0.14 ± 0.05

All values are mean ± SEM. The kinetic constants calculated were from data obtained at six amino acid concentrations (0.002–1 mM) with 10 oocytes per experiment. V_{max}^{app}, K_{app}: kinetic characteristics for amino acid transport measured in the presence of glutamine. Toad 1, 0.05 mM glutamine; Toad 5, 0.1 mM glutamine (V_{max}, K_m: control values.)

order of magnitude (10^{-4} M, 10^{-12} mol/oocyte · min, respectively) as values reported previously by other authors for a variety of amino acids, including alanine (results summarized by Van Winkle, 1988).

Jung et al. (1984*b*) used both radiotracer and electrophysiological methods to demonstrate directly that Na^+/L-alanine cotransport occurred in *Xenopus* oocytes with a net transport stoichiometry of 2 Na^+ : 1 alanine. The present results indicate that oocytes take up both L-glutamine and L-alanine *via* the same Na-dependent transport system, and our Na^+ substitution studies are consistent with the conclusion (Jung et al., 1984*b*) that this system requires 2 Na^+ for inward transport of one neutral amino acid.

Bravo et al. (1976) reported that *Xenopus* oocytes appeared to have distinct systems for the transport of neutral aliphatic, aromatic, basic (cationic) and acidic (anionic) amino acids, but our present results are inconsistent with this conclusion. Those authors reported that uptake of L-alanine (which we believe is transported by the same system as L-glutamine) was inhibited by other aliphatic amino acids (leucine, valine, glycine) but not by arginine or glutamate. In contradiction we have found that glutamine showed mutual transport inhibition with L-arginine, L-glutamate and L-phenylalanine, and that L-alanine uptake was inhibited by arginine (although we have not fully established them all to be substrates of the same transporter). The reasons for this discrepancy are not immediately clear, although it should be noted that Bravo et al. (1976) measured amino acid uptake over periods of 1 to 5 hr, in contrast to our shorter (20 min) uptake period. We have also shown that transport of L-valine (and L-phenylalanine) into oocytes is mainly Na^+-dependent but that of L-glutamate is not. It is known that L-leucine transport in oocytes is Na^+-dependent (Belle, Marot & Ozon, 1976); therefore, it is not unreasonable to propose

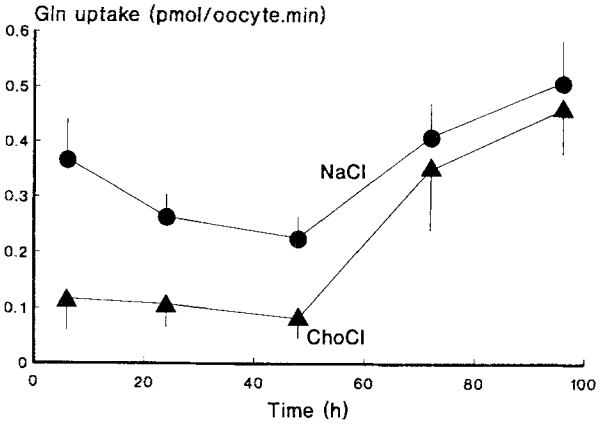

Fig. 6. Temporal changes in L-glutamine transport of *Xenopus* oocytes during culture in Barth's medium (50 μM glutamine; n = 4 toads)

that most, if not all, cationic and (aliphatic) neutral amino acids enter oocytes by the same Na-dependent mechanism.

Which Na^+-dependent amino acid transport system is responsible for L-glutamine uptake in oocytes? Certain defined characteristics of System A (MeAIB-sensitive), System ASC (high stereospecificity for alanine), System N (Li^+-for Na^+ tolerance) and System Gly (glycine and sarcosine only substrates) appear to rule them out as the transport agency (e.g., Christensen & Kilberg, 1987, for review). A stronger candidate appears to be the Na^+-dependent amino acid transporter of broad scope characterized in early embryonic stages of sea urchin and mouse (*cf.* unfertilized oocytes), and denoted System $B^{0,+}$ (Van Winkle, Christensen & Campione, 1985). The similarities between known characteristics of System $B^{0,+}$ (Van Winkle et al., 1985; Van Winkle, 1988) and the oocyte transporter are striking:

a) Both appear to be extremely broad scope and have K_m about 10^{-4} M.
b) Both appear to transport cationic amino acids such as arginine by a Na^+-dependent mechanism.
c) Both appear to require external chloride ions for full activation of amino acid transport.
d) Amino acid transport by both transport systems is inhibited by D-alanine and by bicyclic amino acids such as BCH (BCH at least appears to be a substrate for System $B^{0,+}$).
e) Both have been reported to require two external Na^+ for transport of one neutral amino acid when Na^+ is replaced by choline.
f) Neither appear to tolerate Li^+ for Na^+ substitution.

Our results are therefore broadly consistent with the idea that the major Na^+/amino acid transporter of *Xenopus* oocytes is homologous with System $B^{0,+}$, as speculated by Van Winkle (1988), but we cannot exclude the possibility that the oocytes possess a novel Na^+/amino acid transporter. One possible difference between System $B^{0,+}$ and the oocyte glutamine transporter lies in the apparent sensitivity of System $B^{0,+}$ to choline: System $B^{0,+}$ activity in mouse blastocysts has been reported (*see* Van Winkle, 1988) to be inhibited by choline to such an extent that the results of choline for Na substitution experiments (which indicate that 2 Na^+ are required per amino acid transported) are misleading, and the authors concluded that the true requirement (measured using Li^+ for Na^+ substitution) is 1 Na^+ per amino acid transported. In the case of the oocyte transporter we find that both choline and Li^+, when used as Na^+ replacements, produce results consistent with a requirement for 2 Na^+ per neutral amino acid transported.

During maturation of full-grown oocytes, transport capacity for amino acids decreases, e.g., V_{max} for L-alanine transport in metaphase-arrested oocytes is less than 2% of that in prophase-arrested oocytes (Jung & Richter, 1983). L-Alanine and L-glutamine were transported at rates similar to one another (by Na^+-dependent mechanisms) in metaphase-arrested oocytes.

The glutamine transporter in *Xenopus* oocytes will not tolerate Li^+ as a substitute ion for Na^+, unlike certain other Na^+/amino acid transporters such as System N (Kilberg, Handlogten & Christensen, 1980; Jacob, Rosenthal & Barrett, 1986). This and other distinctive properties of the oocyte glutamine transporter (e.g., strong inhibition by D-alanine and L-arginine) may be exploited to allow the identification, after expression in the oocytes, of exogenous Na^+-dependent glutamine transport systems. It is important to note, however, that culture of oocytes in Barth's medium results in marked changes in the type of endogenous amino acid-transport activity expressed over a period of days and in the degree of this expression over much shorter periods. Our observation that the rate of amino acid transport into oocytes may alter within the first 24 hr of culture has been noted previously (Hallberg & Smith, 1976; Jung et al., 1984*b*).

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