Nonradioactive Monitoring of Organic and Inorganic Solute Transport into Single *Xenopus* Oocytes by Capillary Zone Electrophoresis

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ABSTRACT Transport of organic and inorganic solutes into and out of cells requires specialized transport proteins. Given a sufficiently sensitive analytical method for measuring cellular solute concentrations, it should be possible to monitor solute transport across the plasma membrane at the level of single cells. We report a capillary zone electrophoresis approach that is generally applicable to monitor solute transport into *Xenopus laevis* oocytes, requires only nanoliters of sample, and involves no radioactive materials. The sensitivity of capillary electrophoresis with UV detection is typically on the order of 10^{-5}-10^{-6} M, resulting in the mass detection limits in the low femtomole range. We show that capillary zone electrophoresis serves as a simple technique to measure solute transport into oocytes. Studies of the mammalian oligopeptide transporter PepT1 and the Na^+- and K^+-coupled epithelial and neuronal glutamate transporter EAAC1 expressed in oocytes demonstrate that transport of the dipeptide Trp-Gly via PepT1 and transport of Na^+ and K^+ via EAAC1 across the oocyte plasma membrane can be monitored by measuring intracellular tryptophan absorption and by indirect UV detection of inorganic ions, respectively. The CZE method allowed the simultaneous detection of changes of intracellular Na^+ and K^+ concentrations in response to EAAC1-mediated Na^+ cotransport and K^+ countertransport. This is the first report of a capillary zone electrophoresis-based quantitative analysis of intracellular components of a single cell in response to transport activity.

INTRODUCTION

Changes in the biological, chemical, and physical environment of a cell are often reflected by changes in the concentration of solutes within the cell. Analysis of minute quantities of organic and inorganic solutes of cellular samples at the level of single cells is of broad interest. However, a major hindrance has been the availability of sufficient quantities of biological samples suitable for detection. In this paper we report the use of capillary zone electrophoresis (CZE) for determination of cellular concentrations of organic and inorganic solutes in a single cell, the *Xenopus laevis* oocyte. Using this method, it is possible to monitor transport of organic and inorganic solutes into oocytes, as induced by expression of solute transporters, such as the mammalian H^+-coupled oligopeptide transporter PepT1 (Fei et al., 1994; for a review, see Nussberger and Hediger, 1995) or the Na^+- and K^+-coupled epithelial and neuronal high-affinity glutamate transporter EAAC1 (Kanai and Hediger, 1992; for a review, see Kanai et al., 1993; Hediger et al., 1995).

Capillary zone electrophoresis has recently been shown to be applicable to single-cell monitoring of intracellular constituents of human erythrocytes (Hogan and Yeung, 1992; Xue and Yeung, 1994), neurons from the land snail *Helix aspersa* (for a review, see Kennedy et al., 1989), and adrenal medullary cells (Chang and Yeung, 1995). These studies provided information on cellular solutes, such as sodium, potassium, organic acids, peptides such as glutathione, catecholamines, and neurotransmitters such as dopamine or serotonin. The detection schemes used were based on direct and indirect fluorescence, laser-induced fluorescence, and voltammetry. Highly sensitive detection of organic compounds was accomplished by microderivatization with fluorescent reagents. However, a quantitative analysis of intracellular components using CZE in response to external cellular stimulation and transport protein activity has not yet been reported.

Common methods in use for determining transport properties of recombinant transporters expressed in *Xenopus* oocytes are generally based on the uptake of radioactive isotopes or radioactively labeled compounds, or rely on the electrogenic properties of the transporters, which allow application of electrophysiology techniques such as a two electrode voltage clamp for recordings of whole oocyte currents. The classical nonradioactive methods that allowed the determination of specific solutes at a single cell level have included measurements using ion-selective microelectrodes (Bouvier et al., 1992), or were based on fluorescence microscopy (Blatter and Wier, 1990). The accuracy of these methods, however, may suffer from interfering compounds in the cell. The quantitative analysis of organic compounds in single cells by a separation method remained largely elusive.

Capillary electrophoresis allows rapid (typically within minutes), efficient separation of minute quantities (nanoliter injection volumes, picomoles of substrate) of samples such as nucleic acids, amino acids, peptides, proteins, polysaccharides, and inorganic ions (Karger and Foret, 1993; Foret

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et al., 1993). Capillary electrophoresis measures the mobility of charged particles under the influence of an electric field, and the observed migration time of a species is determined by a combination of its electrophoretic mobility and the velocity of electroosmotic flow. In this study we have used on-column UV detection in both direct (detection of UV absorbing tryptophan) and indirect (UV-absorbing background electrolyte) modes (Foret et al., 1989, 1990).

MATERIALS AND METHODS

Materials

All chemicals used for the CZE experiments in this study were of analytical grade and were purchased from Sigma Chemical Co. if not otherwise specified.

Xenopus oocyte expression of human EAAC1 and rabbit PepT1

Human EAAC1 and rabbit PepT1 were expressed in oocytes as recently described (Kanai et al., 1994; Fei et al., 1994). Briefly, complementary RNA (cRNA) of human EAAC1 and rabbit PepT1 were obtained by in vitro transcription from cDNAs in pBluescript SK^- (Stratagene) using T7 RNA polymerase (Pharmacia). Collagenase-treated (collagenase A; Boehringer Mannheim) and manually defolliculated oocytes were injected with 10 ng cRNA per oocyte the day after defolliculation and maintained in modified Barth's medium (88 mM NaCl, 1 mM KCl, 0.33 mM $Ca(NO_3)_2$, 0.41 mM $CaCl_2$, 0.82 mM $MgSO_4$, 2.4 mM $NaHCO_3$, 10 mM HEPES, pH 7.4) supplemented with 50 μg/ml gentamicin at 18°C. Control oocytes were injected with 50 nl deionized water. Oocytes were used 4 to 7 days after injection.

Uptake experiments

To confirm that PepT1 and EAAC1 were functionally expressed in oocytes individual oocytes were tested using the two-electrode voltage clamp technique to measure substrate (1 mM Trp-Gly, 1 mM Trp-Glu, or 1 mM L-Glu)-evoked currents as previously described (Kanai et al., 1994; Fei et al., 1994). PepT1 cRNA- and water-injected oocytes were then incubated in uptake solution (100 mM NaCl, 2 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 3 mM HEPES, 3 mM MES, 3 mM Tris, pH 5.5) in the presence of 1 mM Trp-Gly for 10 min, 35 min, 60 min, and 2 h 30 min or in the presence of 1 mM Trp-Glu for 60 min. Oocytes were then washed with ice-cold substrate-free uptake medium in which the pH was adjusted to pH 7.5 to remove external substrate, quickly frozen, and stored at −20°C. Human EAAC1 cRNA-injected, noninjected, and water-injected control oocytes were first incubated for 30 min in Na^+-free uptake solution containing 100 mM choline-Cl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.4, in the presence of 1 mM ouabain, 0.1 mM amiloride, and 0.1 mM bumetanide to inhibit endogenous Na^+ transport. Uptake experiments were performed in standard uptake medium (ND96) containing 96 mM NaCl, 2 mM KCl, 1.8 mM $CaCl_2$, 1 mM $MgCl_2$, and 5 mM HEPES, pH 7.4, in the presence of 1 mM L-glutamate and inhibitors (ouabain, amiloride, and bumetanide). After 1 h of incubation, individual oocytes were washed in ice-cold Na^+/K^+-free washing solution (200 mM mannitol, 2 mM $CaCl_2$, 2 mM $MgCl_2$, 1 mM ouabain, 0.1 mM amiloride, 0.1 mM bumetanide) and kept at −20°C. Aliquots of the individual washing solutions were kept for CZE analysis and atomic emission spectrometry and were used to confirm that any residual extracellular substrate had been removed.

Oocyte processing

Single oocytes were homogenized in 100 μl deionized water (Life Technologies, Gaithersburg, MD) and boiled for 5 min to inactivate proteases and peptidases. The oocyte lysate was centrifuged in a benchtop microcentrifuge at 14,000 rpm for about 40 min to pellet yolk protein and membranous elements. After centrifugation, an aliquot of the supernatant of EAAC1-injected oocytes was transferred into a new tube and stored at −20°C. For PepT1-injected oocytes another 100 μl of deionized water was added to the supernatant, and high-molecular-weight compounds were removed by ultrafiltration using Amicon microcon filters (molecular weight cut-off of 3000; Amicon, Beverly, MA). The filtrate was dried in a speed evacuator, resuspended in 3–5 μl deionized water, and stored at −20°C. To estimate the amount of dipeptides transported into PepT1-injected oocytes using the CZE method, ferroin was added as an internal standard. Accordingly, the resuspended filtrate was mixed with ferroin standard solution (OD_{268} = 1.65) containing 5 mM Na_2HPO_4/H_3PO_4, pH 2.3, at a ratio of 1:1. To determine the absolute concentrations of Na^+ and K^+ in the oocytes using CZE, an equal volume of RbCl (500 μM) was added to the supernatant of EAAC1-injected oocytes as an internal standard. For validation of the capillary electrophoresis method, the determination of intracellular Na^+ and K^+ was also performed by atomic emission spectrometry. Because this method is considerably less sensitive, pools of 10 oocytes were washed in Na^+/K^+-free washing solution (see above) and were processed in the same way as PepT1 cRNA-injected oocytes, with the only modification being that the filtrate was dissolved in 25 μl deionized water. A 20-μl aliquot of this solution was diluted 100-fold in 1.5 mM CsCl and subjected to atomic emission spectrometry (IL943 atomic flame photometer; Instrumentation Laboratory, Lexington, MA). The remaining solution was directly used for measuring Na^+ and K^+ using CZE. The atomic flame photometer was calibrated using test solutions with $[Na^+]$ and $[K^+]$ varying from 0 to 10 mM.

Instrumentation

The capillary electrophoresis system used in this study was a laboratory-made unit equipped with a data acquisition system for chromatography (Chrom Perfect; Justice Innovations, Mountain View, CA). The capillary tubing was of uncoated silica with an inner diameter of 75 μm (Polymicro Technologies, Phoenix, AZ), a total length of 34–40 cm, and a length from inlet to detector (Spectra 100 UV, TSP, Freemont, CA) of 28–30 cm. The experiments were performed at a constant voltage of 10–30 kV using a CZE 1000 R high-voltage power supply (Spellman, Plainview, NY). The currents were 10–30 μA.

Determination of intracellular Na^+, K^+, and tryptophan by capillary electrophoresis

Electrolytes used for the separation by CZE, direct detection of the dipeptides Trp-Gly and Trp-Glu, and indirect detection of Na^+ and K^+ were 50 mM Na_2HPO_4/H_3PO_4, pH 2.3, and 20 mM imidazole/acetic acid, pH 4.8, respectively. The detection wavelength for tryptophan was 280 nm, and that used for indirect detection of inorganic ions was 215 nm. Before injection, the capillary tubing was rinsed with 4 M NaOH, deionized water, and separation buffer. Injection was performed by gravity (Δh = 10 cm), and samples were introduced by dipping the end of the capillary tube into a small vial containing the sample. The volume and duration of injection were 8–24 nl and 5–30 s. Intracellular tryptophan, Na^+, and K^+ accumulation was quantitated by integration of the corresponding peak areas A_{Trp}, A_{Na}, and A_K of the electropherograms using the peak areas of the internal standards ferroin (A_{Fer}) and Rb^+ (A_{Rb}) to correct for injection volume errors. The CZE system was calibrated using test solutions, with [Trp] varying from 0 to 250 μM and $[Na^+]$ and $[K^+]$ varying from 0 to 500 μM. Ferroin (OD_{268} = 0.825) and Rb^+ (250 μM) were used as internal standards, respectively. There was a linear increase of the absorption ratios of A_{Trp}/A_{Fer}, A_{Na}/A_{Rb}, and A_K/A_{Rb} with increasing Trp, Na^+, and K^+

concentrations, respectively. The absolute concentrations of tryptophan, Na^+, or K^+ in a given sample were computed using the following equations:

$$[\mathrm{Trp}] = \frac{1}{b_{\mathrm{Trp}}} \times \frac{A_{\mathrm{Trp}}}{A_{\mathrm{Fer}}}; \qquad [\mathrm{Na}^+] = \frac{1}{b_{\mathrm{Na}}} \times \frac{A_{\mathrm{Na}}}{A_{\mathrm{Rb}}};$$

$$[\mathrm{K}^+] = \frac{1}{b_{\mathrm{K}}} \times \frac{A_{\mathrm{K}}}{A_{\mathrm{Rb}}};$$

where b_{Trp}, b_{Na}, and b_{K} are the slopes of the corresponding calibration curves. In our CZE setup, b_{Trp}, b_{Na}, and b_{K} were 0.04 μM^{-1} (r^2 = 0.99), 7.54×10^{-3} μM^{-1} (r^2 = 0.98), and 4.89×10^{-3} μM^{-1} (r^2 = 0.99), respectively. The relative response R_{corr} of Na^+ and K^+ using the CZE method is given by the equation

$$A_{\mathrm{K}}/A_{\mathrm{Na}} * R_{\mathrm{corr}} = [\mathrm{K}^+]/[\mathrm{Na}^+];$$

where $R_{\mathrm{corr}} = b_{\mathrm{Na}}/b_{\mathrm{K}}$. The relative response R_{corr} was 1.54.

RESULTS AND DISCUSSION

We have studied the cellular accumulation or extrusion of Na^+ and K^+ and of the dipeptides Trp-Gly and Trp-Glu using the CZE method (Fig. 1). Based on studies of oligopeptide transport (PepT1) and high-affinity glutamate transport (EAAC1), we demonstrate that the method is suitable to monitor carrier mediated uptake of these solutes across the plasma membrane of *Xenopus* oocytes.

Oligopeptide transport via PepT1

Transport of small peptides across biological membranes plays a central role in a wide spectrum of cellular processes.

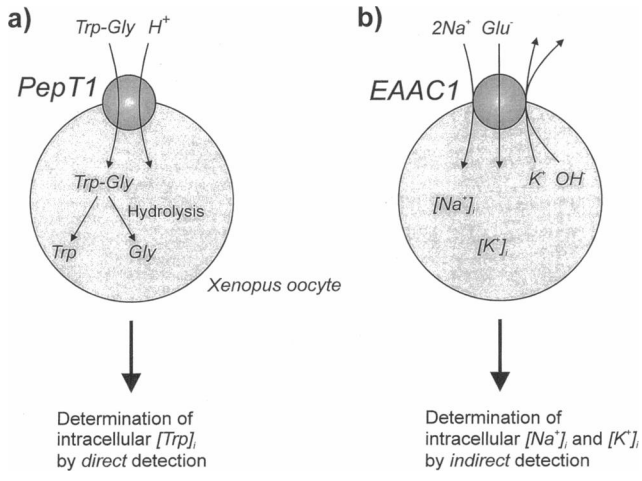

FIGURE 1 Transport properties of PepT1 and EAAC1 expressed in *Xenopus* oocyte plasma membranes. (*a*) Cellular uptake of Trp-Gly and Trp-Glu via the oligopeptide transporter PepT1. Inside the oocyte, oligopeptides are rapidly hydrolyzed into single amino acids. The uptake of Trp-Gly and Trp-Glu was monitored in single oocytes by determination of intracellular Trp using CZE in direct detection mode. (*b*) The uptake of glutamate is coupled to the cotransport of two Na^+-ions and the countertransport of K^+. This model is in agreement with the determination of intracellular Na^+ and K^+ using CZE in direct detection mode.

These include the cellular uptake of the end products of protein degradation, the metabolism of peptide hormones, the antigen processing and presentation by major histocompatibility complex molecules, and cellular peptide processing and secretion pathways (for a review, see Nussberger and Hediger, 1995). The cDNA of an important mammalian oligopeptide transporter (PepT1) that mediates epithelial uptake of small peptides such as di-, tri- and tetrapeptides has recently been identified (Fei et al., 1994). When expressed in *Xenopus* oocytes, PepT1 mediates uptake of di- and tripeptides, as well as peptide-like drugs such as β-lactam antibiotics (Fei et al., 1994). PepT1-mediated uptake of the dipeptide glycyl-sarcosine (Gly-Sar) resulted in an intracellular Gly-Sar accumulation of about 3000 pmol per oocyte within 60 min. This amount is detectable by CZE with UV light. We therefore used PepT1 as a model system to monitor uptake of organic substrates into *Xenopus* oocytes (Fig. 1). We have chosen Trp-Gly and Trp-Glu as substrates, because the indole ring of Trp is easily detectable using CZE in direct detection mode at a wavelength of 280 nm. It is likely, however, that the method can also be adapted to the detection of nonaromatic compounds. For example, we have detected dimethylurea, a substrate of the mammalian urea transporter UT2 (You et al., 1993), at 206 nm and were able to separate it from endogenous oocyte components (data not shown).

PepT1-mediated uptake of the dipeptides Trp-Gly or Trp-Glu resulted in intracellular accumulation of tryptophan. Because we were unable to detect intact Trp-Gly or Trp-Glu in oocytes (Fig. 2; data of Trp-Glu uptake not shown) we infer that the absorbed peptides are rapidly degraded into Trp and Gly or Trp and Glu, respectively. Water-injected control oocytes did not show significant Trp-Gly uptake or accumulation of intracellular tryptophan.

The time course of PepT1-mediated uptake of Trp-Gly was then studied. In these experiments, we anticipated an intrinsic variation in the injection volumes of the samples and used an internal standard (ferroin) to compensate for this variation. Accordingly, the electropherograms depicted in Fig. 2 are normalized to the peak area of the standard ferroin ($\sim 10^{-4}$ M). The reproducibility of the migration times of separated substances was on the order of ±10%.* Fig. 3 shows the time course of PepT1-mediated Trp-Gly uptake into single oocytes based on the normalized peak area of tryptophan. The time course was linear over the first ~60 min, consistent with ^{14}C-labeled dipeptide glycyl-sarcosine (Gly-Sar) uptake studies (Fei et al., 1994). This time course is determined by at least two parameters, the initial rate of Trp-Gly uptake via PepT1 and the rate of Trp-Gly hydrolysis. As mentioned above, the dipeptide

* This error is due to the fact that a simple instrument without capillary temperature control was used; because the background electrolytes generate relatively large electric currents, and therefore high Joule heat, relatively large temperature variations can be expected; a temperature change of one degree is sufficient to cause more than a 2% change of the electrophoretic mobilities (Foret et al., 1993).

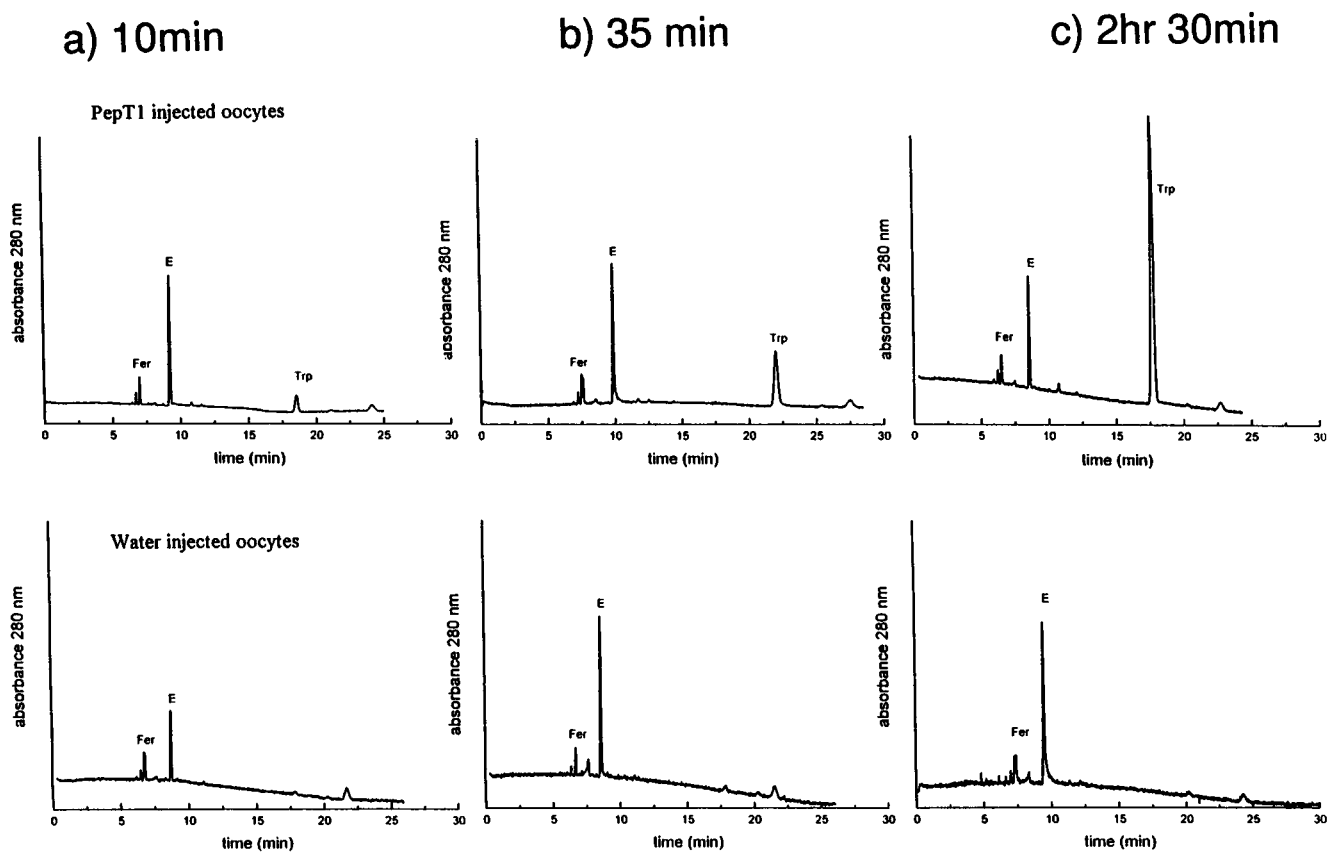

FIGURE 2 PepT1-mediated Trp-Gly uptake. The electropherograms show the result of the filtrate of single PepT1 and water injected oocytes incubated for (*a*) 10 min, (*b*) 35 min, and (*c*) 2 h 30 min in uptake solution. The total electrophoresis time was 30 min at 30 kV, using 280 nm as the detection wavelength. Ferroin (peak Fer) was used as an internal standard. Peak E is a not yet defined endogenous compound present in oocytes. Peak Trp represents the intracellular tryptophan increase with time. This is entirely consistent with an increase of intracellular tryptophan in a single oocyte due to the Trp-Gly transport activity of PepT1 expressed in the oocyte plasma membrane. The tryptophan peaks were identified by spiking test samples with tryptophan.

Trp-Gly was not present at detectable levels in oocytes, indicating that there is rapid peptide degradation by peptidases. This suggests that the transport rate of the dipeptide via PepT1 is rate limiting. It should be noted that Peak E (Fig. 2) does not correspond to the dipeptide Trp-Gly. It represents an endogenous compound of the oocyte that remains to be defined. The peak for Trp-Gly would run at a migration time between peak E and the tryptophan peak (data not shown).

Assuming that tryptophan was not significantly metabolized, the increase of intracellular tryptophan can be assumed to reflect the transport rate of Trp-Gly via PepT1. The time course of the increase of PepT1-mediated uptake of Trp-Gly is consistent with earlier time course experiments using ^{14}C-Gly-Sar as a substrate (Fei et al., 1994), which revealed that equilibrium of PepT1-mediated uptake is reached after ~6 h.

To estimate the absolute amount of PepT1-mediated Trp-Gly uptake into oocytes, Trp-Gly uptake experiments were repeated, using ferroin (OD_{268} = 0.825) as an internal standard to correct for injection volume errors (electropherograms not shown). For oocytes that were incubated with 1 mM Trp-Gly for 2 h 30 min, the concentration of Trp measured in a sample volume of ~3 μl (see Materials and Methods) was 177 ± 11 μM (n = 2), which corresponded to 531 ± 33 pmol (n = 2) Trp per oocyte. This value is in the expected range of PepT1-mediated uptake of various substrates. For example, the uptake of ^{14}C-Gly-Sar was ~1500 pmol/2 h 30 min per oocyte (Fei et al., 1994).

EAAC1-mediated uptake

Glutamate transport is of critical importance for excitatory synaptic transmission, normal cellular function, and epithelial amino acid metabolism. We previously reported the expression cloning of the rabbit intestinal high-affinity glutamate transporter EAAC1 (Kanai and Hediger, 1992; Kanai et al., 1993). Stoichiometric studies indicated that EAAC1-mediated uptake of L-glutamate is coupled to the cotransport of two Na^+ ions, the countertransport of one K^+ and the countertransport of one OH^- ion, or, alternatively, the cotransport of one H^+ ion (Kanai and Hediger, 1992; Kanai et al., 1995). Based on this stoichiometry, EAAC1 can concentrate L-glutamate up to 10,000-fold across cell membranes (Kanai et al., 1995). Given this unique stoichi-

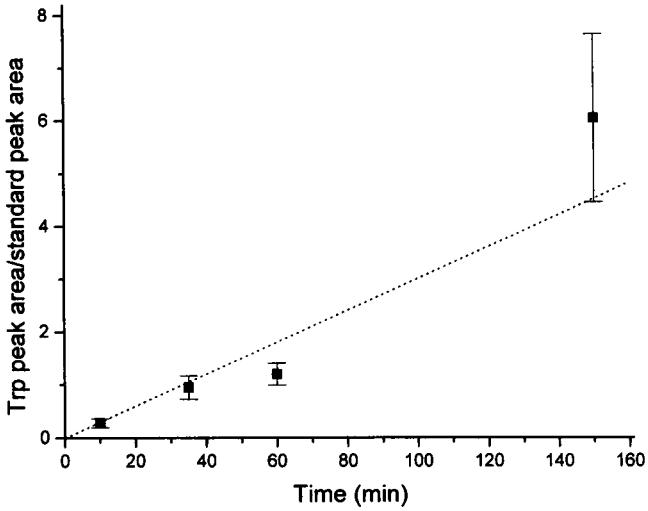

FIGURE 3 Time course of PepT1 mediated uptake of Trp-Gly. Single oocytes were incubated in uptake solution in the presence of 1 mM Trp-Gly. Single oocytes (n = 3; n = 2 for 10 min uptake) were taken at each time point, processed, and subjected to CZE measurements.

ometry, EAAC1 is a challenging protein to test the application of the CZE system to membrane transport studies in *Xenopus* oocytes.

Fig. 4 shows a typical electropherogram of a single, defolliculated noninjected *Xenopus* oocyte. The figure shows that various ions such as Na^+, K^+, Ca^{2+}, and Mg^{2+} can be separated and detected using CZE in indirect detection mode. Rb^+ was added as an internal standard to correct for injection volume errors and as a reference to determine the absolute concentrations of Na^+ and K^+ in the oocytes. It should be noted that the peaks representing Ca^{2+} and Mg^{2+} do not solely reflect the oocyte Ca^{2+} or Mg^{2+} content because of residual ion contaminations that are mostly derived from the washing step with mannitol/Ca^{2+}/Mg^{2+} solution. Nevertheless, the peaks provide a rough estimate of the intracellular concentrations of these divalent ions. Taking into account that the detection of Na^+ by the indirect detection mode was about 1.54-fold more sensitive than that of K^+ (see Materials and Methods), the integrated peak areas of Na^+ and K^+ reveal an intracellular molar Na^+-to-K^+ ratio of 0.29 ± 0.01 (mean ± SEM, n = 9). The absolute ion concentrations of Na^+ and K^+ were 18.00 ± 0.08 nmol (n = 9) and 61.70 ± 2.45 nmol (n = 9) per oocyte or, based on an average oocyte volume of 0.5 μl (Dascal, 1987), 36.0 ± 1.5 mM and 123.4 ± 4.9 mM, respectively. These values are roughly consistent with previous Na^+/K^+ analyses of *Xenopus* oocytes that revealed intracellular concentrations of Na^+ between 6.0 and 22.5 mM and of K^+ between 62 and 150 mM (for review, see Dascal, 1987), yielding a molar Na^+-to-K^+ ratio between 0.04 and 0.36. It is important to note that these values vary among different batches of oocytes, because a slight variation of the oocyte diameter by only 0.1 mm results in a change of the calculated concentration by roughly 28%. Because it is difficult to determine the exact intracellular volume of oocytes, the absolute intracellular concentrations of Na^+ and K^+ cannot be determined with high accuracy. However, the Na^+-to-K^+ ratios can be determined accurately with this method.

In a previous report in which we addressed the concentration of organic solutes in oocytes (Fei et al., 1994), we used an intracellular "active" volume of 0.25 μl. This value was obtained based on the diffusion of ^{14}C-labeled urea via the facilitated urea transporter UT2 (You et al., 1993). In contrast, the herein used value of 0.5 μl, which was calculated based on an average oocyte diameter of 1 mm, appears

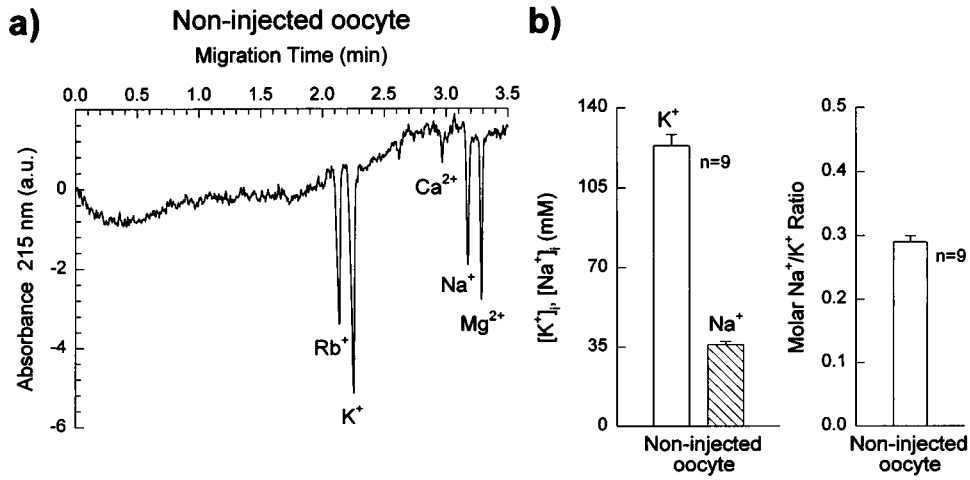

FIGURE 4 Separation of intracellular inorganic ions. (*a*) The electropherogram was obtained from one noninjected oocyte. The oocyte was dissolved in 100 μl deionized water. After centrifugation, an aliquot of the supernatant was mixed with an equal volume of RbCl (500 μM), used as internal standard to correct for injection volume errors, and subjected to CZE analysis. The separation was driven by a 10-kV potential in a 10 mM imidazole, pH 4.8 buffer. The detection wavelength was 215 nm. (*b*) Based on an average oocyte volume of 0.5 μl and given that the response of Na^+ is 1.54-fold of that of K^+, the molar Na^+ to K^+ ratio was derived from the corresponding integrated peak areas. The absolute ion concentrations were determined by comparison of the peak areas of Na^+ and K^+ with CZE experiments of known Na^+/K^+ concentrations.

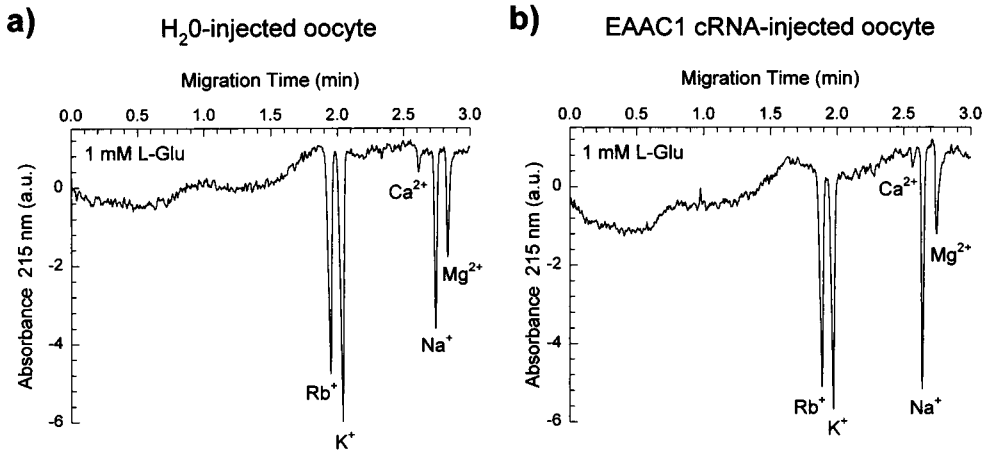

FIGURE 5 Na^+/K^+-coupled glutamate transport via EAAC1. Electropherograms of water (*a*) and EAAC1 (*b*) cRNA injected oocytes incubated (60 min) in 1 mM L-glutamate in ND96 uptake solution. Ouabain, amiloride, and bumetanide were added to inhibit endogenous Na^+ and K^+ transport. The electropherograms represent a single water and EAAC1 injected oocyte each. Oocytes expressing EAAC1 showed large glutamate-induced Na^+ uptakes in the presence of 1 mM L-glutamate. Given that the response of K^+ ions is 1.54 times less sensitive than that of Na^+ ions, the comparison of the K^+ peaks of water and EAAC1 injected oocytes shows that EAAC1-mediated glutamate transport is associated with the countertransport of K^+ ions. As in Fig. 4, the peak areas of Rb^+ correspond to 250 μM Rb^+.

to be more appropriate for small inorganic ions such as Na^+ and K^+. It is possible that organic solutes such as urea and peptides are distributed in a smaller active volume in oocytes than in small inorganic ions.

Typical electropherograms of water- and EAAC1-injected oocytes are shown in Fig. 5. A comparison of the absolute ion concentrations and the molar ratio of Na^+ to K^+ obtained from single EAAC1 cRNA-injected, water-injected, or noninjected oocytes based on CZE is presented in Fig. 6. These experiments represent the result from a 1-h incubation in standard ND96 uptake solution in the presence of 1 mM L-glutamate. Ouabain, amiloride, and bumetanide were added to the bath to inhibit endogenous Na^+ and K^+ transport. In these CZE experiments, the molar Na^+-to-K^+

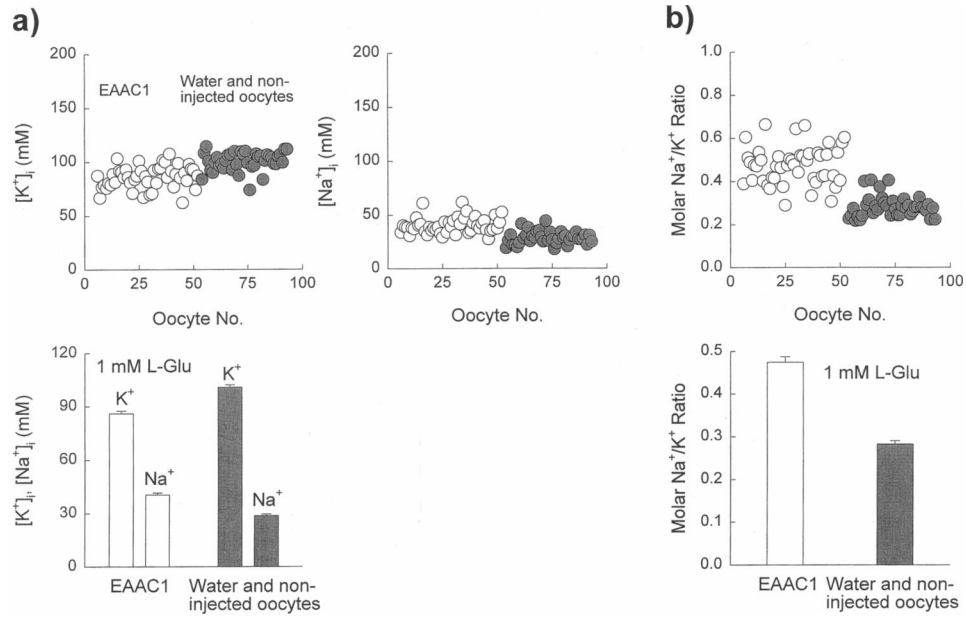

FIGURE 6 Na^+ to K^+ ratio and Na^+/K^+ concentration. (*a*) Comparison of the concentrations of Na^+ and K^+ in EAAC1 cRNA and water/noninjected oocytes reveals an increase in intracellular Na^+ and a decrease in intracellular K^+ in the presence of 1 mM L-glutamate (1 h incubation). The ion concentrations were calculated from the corresponding peak areas of electropherograms as depicted in Fig. 5, using the peak area of Rb^+ as reference to correct for injection volume errors. (b) The Na^+/K^+ ratio was 0.28 ± 0.01 (mean ± SEM, n = 40) for water injected oocytes and 0.47 ± 0.01 (mean ± SEM, n = 47) for oocytes expressing EAAC1. The data confirm that EAAC1-mediated glutamate transport is coupled to the cotransport of Na^+ ions and the countertransport of K^+ ions. Oocytes no. 11-52, no. 54-71, and no. 72-93 represent EAAC1 cRNA-injected, water-injected, and noninjected oocytes, respectively.

ratios of water-injected/noninjected oocytes and of EAAC1-injected oocytes were 0.28 ± 0.01 (mean ± SEM, n = 40) and 0.47 ± 0.01 (n = 47), respectively (Fig. 6). The K^+ and Na^+ concentrations, based on an oocyte volume of 0.5 μl, were 100.7 ± 1.4 mM (n = 40) and 28.6 ± 1.0 mM (n = 40), respectively, for water-injected/noninjected oocytes, and 86.0 ± 1.5 mM (n = 47) and 40.4 ± 1.1 mM (n = 47), respectively, for EAAC1-injected oocytes. The increase of the intracellular concentration of Na^+ by 12 mM as well as the decrease of the intracellular K^+ concentration by about 15 mM is consistent with the view that high-affinity glutamate transporters are coupled to the cotransport of Na^+ and the countertransport of K^+ (Kanai and Hediger, 1992; Bouvier et al., 1992). This is the first direct demonstration that K^+ exits the cell in response to glutamate uptake via a cloned high affinity glutamate transporter.

Given that the uptake of glutamate by EAAC1 reaches its equilibrium after ~1 h, the increase of the intracellular concentration of Na^+ is also consistent with recent studies involving $^{22}Na^+$ uptake studies by EAAC1 expressed in oocytes in the presence of 1 mM L-glutamate (Kanai et al., 1995). These studies gave $^{22}Na^+$ uptakes of about 3000 pmol per oocyte per 20 min, whereas water-injected control oocytes did not show significant $^{22}Na^+$ uptake. Assuming an oocyte volume of 0.5 μl, 3000 pmol corresponds to an increase in $[Na^+]_i$ of roughly 6 mM. This value is in line with the 12 mM increase obtained after a 1h uptake based on CZE. CZE therefore offers a suitable nonradioactive approach to studying Na^+ transport via recombinant transport proteins. A striking observation was that the amount of K^+ that exits the cells was approximately the same as the Na^+ uptake. This finding is inconsistent with the stoichiometry of EAAC1, which was proposed to involve coupling of glutamate uptake to the cotransport of 2 Na^+ ions and the countertransport of 1 K^+ ion. It should be noted, however, that the uptake studies were not performed under voltage clamp condition. Because one positive charge is entering the cell on EAAC1 with each glutamate molecule, a positive charge has to leave the oocyte again. This charge most likely leaves as a K^+ ion through K^+ channels. This would predict that the decrease of intracellular K^+ should be exactly equal to the increase of intracellular Na^+, as was experimentally demonstrated.

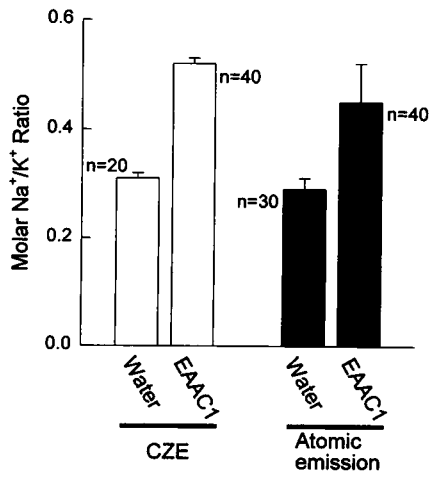

FIGURE 7 Na^+ to K^+ ratio determined by CZE and atomic emission spectrometry. Pools of 10 EAAC1 cRNA and 10 water-injected oocytes incubated in ND96 uptake solution and 1 mM L-glutamate for 1 h were subjected to CZE and atomic emission spectrometry.

When optimizing a new approach, it is always useful to compare its accuracy with that of previously used techniques. Fig. 7 shows the relative molar ratios of Na^+ to K^+ obtained from pools of 10 oocytes that were injected with EAAC1 cRNA or H_2O. The figure compares the ratios determined based on atomic emission spectrometry or CZE. Both experiments represent the result from 1-h incubations in standard ND96 uptake solution containing 1 mM L-glutamate. In the CZE experiments, the molar Na^+-to-K^+ ratios of water and EAAC1-injected oocytes were 0.31 ± 0.01 (n = 4 × 10) and 0.52 ± 0.01 (n = 2 × 10), respectively. Consistent with these results, atomic emission spectrometry revealed molar Na^+-to-K^+ ratios of 0.29 ± 0.02 (n = 3 × 10) and 0.45 ± 0.07 (n = 4 × 10) for water- and EAAC1-injected oocytes, respectively.

The intracellular concentration of Na^+, as determined by atomic emission spectrometry, was 26.0 ± 2.1 mM for water-injected oocytes and 39.0 ± 2.4 mM for oocytes expressing the human high-affinity glutamate transporter EAAC1. The value of the intracellular K^+ concentration of water-injected oocytes was 88.6 ± 1.2 mM and 86.8 ± 6.1 mM of EAAC1-injected oocytes. In general, the results obtained with CZE and atomic emission spectrometry are therefore similar. The advantages of the CZE methods are 1) higher sensitivity so that single cells can be analyzed, 2) several ions can be monitored simultaneously, and 3) both organic and inorganic ions can be analyzed.

In conclusion, CZE in combination with the *Xenopus* oocyte expression system provides a versatile method for studying the transport activity of solute transporters expressed in *Xenopus* oocytes and to monitor transport in a quantitative manner without the need for radioisotopes. Low sample volumes required for CZE analysis (a few nanoliters) combined with its good concentration detection limit (~10^{-5}-10^{-6} M) allow quantitative determination of substances in single oocytes. In principle, CZE is also applicable to the detection of changes of intracellular substrates. Future work may provide further applications, for example, studies involving other organic solute transporters, or inorganic ion transporters, such as members of the electroneutral Na^+-(K^+)-Cl^- cotransporter family (Gamba et al., 1994; Delpire et al., 1994).

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