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Rapid Report

Multiple components of transport are associated with murine cationic amino acid transporter (mCAT) expression in *Xenopus* oocytes

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Abstract

Expression of putative amino acid transport proteins is usually assumed to be associated with expression of a single component of transport. It is shown in this report, however, that murine cationic amino acid transporter (mCAT) expression in *Xenopus* oocytes is associated in important instances with expression of more than one kinetically distinguishable transport process. Accurate knowledge of the kinetics of transport continues, therefore, to be needed to understand how transport proteins function.

Keywords: Amino acid; Transport protein; Transporter; Oocyte; Expression cloning; (*Xenopus*)

Amino acid transport systems catalyze movement of amino acids across cellular membranes, but unlike enzymes, they do not destabilize their substrates. Although transport through some intracellular membranes has been well-characterized, transport has been studied most widely in the plasma membrane [1]. The number of recognized amino acid transport systems has increased considerably since the A and L systems were distinguished over thirty years ago [2]. These systems have been classified according to whether they are Na^+-dependent or Na^+-independent and whether they prefer anionic, cationic or zwitterionic substrates, although some systems do not fit well into this conventional classification scheme [3].

Different amino acid transport systems are currently believed to arise in at least three ways. They can, of course, arise from expression of different genes that encode different transport proteins [4]. In addition, alternative splicing of mRNA leads to somewhat different transport proteins which may have the same [5] or different [6–8] transport characteristics. Finally, some transport systems appear to be composed of a transport protein and one or more accessory proteins [4]. The multiple components of transport associated with expression of the putative accessory protein, rBAT [4], presumably result from complexes of this protein with at least two different endogenous transport proteins in *Xenopus* oocytes. Similarly, the same transport protein can probably become a component of different systems by associating with different accessory proteins, although specific instances of this phenomenon have not as yet been documented.

Recently, cDNAs encoding numerous putative amino acid transport proteins have been isolated and their cRNAs expressed in *Xenopus* oocytes and other cells (reviewed in Ref. [4]). A few of the proteins have also been purified and reconstituted in liposomes, thus, verifying their membrane transport activity [4]. In all of these cases, a single component of amino acid transport apparently has been assumed to be associated with expression of the proteins. It is also possible, however, that the protein can associate with different endogenous accessory proteins or that it can exist in different stable conformations, which could give rise to multiple activities with different transport characteristics. In studies reported here, we explored the possibility that more than one kinetically distinct component of transport may be associated with expression of the murine cationic amino acid transporters (mCATs) in *Xenopus* oocytes.

MCAT-2 [9,10] and mCAT-1 (gift from Dr. James Cunningham) cDNAs were directionally subcloned into the pSP72 plasmid (Promega) so that the 5′ termini were adjacent to the SP6 promoter for in vitro transcription. The purified cRNAs were dissolved in DEPC-treated water at

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1.0–4.0 mg/ml and injected into *Xenopus* oocytes as previously described [6]. Control oocytes from the same batch of cells as the test oocytes were treated in the same ways except that they were injected with either 50 nl of water or 50 nl of water containing GluR3 cRNA (gift of Stephen Heinemann) [11]. Oocytes with an initial membrane potential of at least −45 mV were selected for use in transport studies.

Currents that resulted from amino acid transport were monitored in oocytes under voltage-clamp conditions at −60 mV as previously described [6]. Recordings were made in gentamicin and pyruvate-free Barth's solution. Amino acids were dissolved in the recording solution at pH 7.3 and applied by bath exchange using a constant-flow recording chamber. Transport by individual oocytes was studied at several different substrate concentrations as indicated in the figures. Oocytes were exposed to each amino acid at different concentrations for 30 s preceded and followed by washes with amino acid free Barth's solution.

Use of the same oocyte to study electrogenic transport at several different substrate concentrations can be more efficient and reliable for kinetic studies than measuring transport of radiolabeled amino acids [12]. When transport of a radiolabeled substrate is measured, an oocyte can be studied at only one substrate concentration, so uptake needs to be measured in enough oocytes at each substrate concentration to obviate considerable variability in uptake by different oocytes expressing an mCAT protein. A significant amount of the variability in this electrogenic uptake among oocytes probably results from differences in resting membrane potential, and this variability is eliminated under voltage-clamp conditions [8,12]. Further characterization of the substrate selectivities of each of two or more components of transport that have been detected utilizing electrical measurements would, of course, require the use of radiolabeled substrates; amino acid analogs used to inhibit one or more of the components would themselves be likely to be electrogenic [4].

In addition to studies of mCAT-1 and mCAT-2, we used data reported by Kavanaugh and associates [8] to assess transport associated with expression of mCAT-2a in *Xenopus* oocytes. The mCAT-2 gene encodes two proteins that are products of alternative splicing [13]. The mCAT-2 protein has been studied in three separate laboratories and reportedly catalyzes high-affinity transport [6,8,14], whereas mCAT-2a has been reported by two of these laboratories to have much lower affinity for its substrates [7,8,14].

The mediated L-arginine uptake associated with either mCAT-1 or mCAT-2 expression in *Xenopus* oocytes was assessed in Hofstee plots. This analysis is consistent with at least two kinetically distinct transport processes (Fig. 1). Similarly curved Hofstee plots were obtained for the transport of L-ornithine, L-lysine and L-histidine that resulted from expression of mCAT-2 in *Xenopus* oocytes (data not shown). In addition, reassessment (Fig. 2) of data reported elsewhere (Fig. 2 of Ref. [8]) revealed two components for the L-arginine transport that resulted from mCAT-2a expression in *Xenopus* oocytes (referred to as CAT2α in Ref. [8]). A curved Hofstee plot for uptake of radiolabeled arginine by *Xenopus* oocytes expressing mCAT-2a has also been presented in another study, although the data were assumed to represent a straight line for purposes of that analysis [7].

In contrast to results reported here for mCAT-2 (Fig. 1), reassessment of data for mCAT-2 (CAT2β) reported elsewhere [8] was consistent with expression of a single

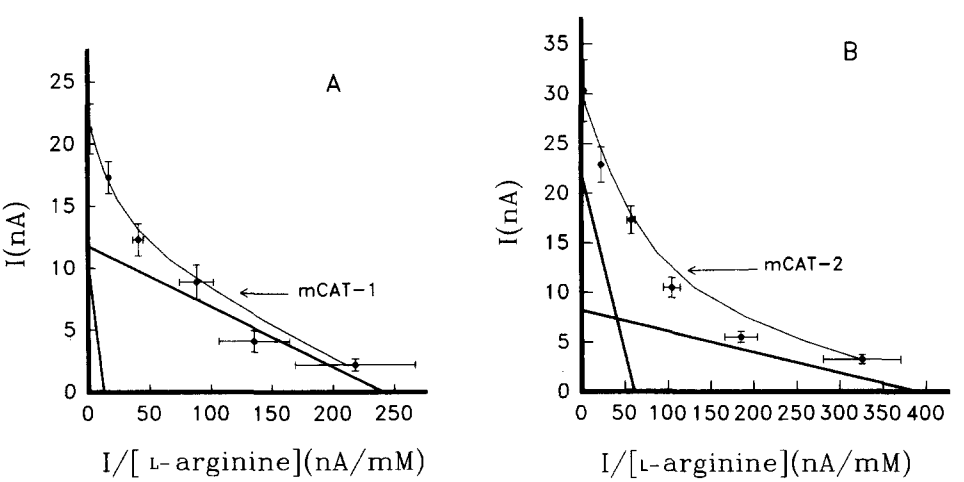

Fig. 1. Hofstee plots of the currents induced by L-arginine transport in *Xenopus* oocytes expressing mCAT-1 (A) or mCAT-2 (B). The currents induced by transport were measured at 0.01–10.0 mM L-arginine in 11 (A) or 15 (B) oocytes, and the mean ± S.E. currents were calculated for each concentration. The current induced by substrate in control (water injected) oocytes was only about 7% of that in oocytes expressing an mCAT protein. These control values, which included nonsaturable uptake, were deducted to produce the data shown. Mediated arginine transport was resolved into two components using the method of Spears et al. [15]. The straight lines represent these components and the curved lines represent the combinations of these straight lines. The K_m values determined in this way were about 49 and 860 μM for mCAT-1 and about 21 and 360 μM for mCAT-2.

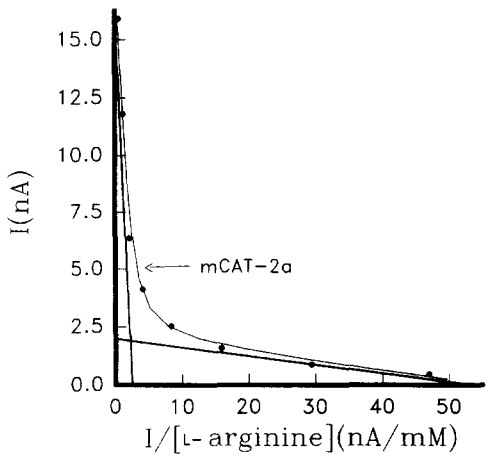

Fig. 2. Hofstee plot of the current induced by arginine transport in *Xenopus* oocytes expressing MCAT-2a (CAT2α). The data were taken from Fig. 2 of Ref. [8] after substraction of control values for a vehicle-injected oocyte in the same figure. Transport was resolved into two components with K_m values of about 37 μM and 6.6 mM [15]. The two straight lines represent these two components of transport, and the curved line represents the combination of the two straight lines.

component of transport in *Xenopus* oocytes (not shown). It is conceivable that our results for mCAT-2 (Fig. 1) differ from those reported elsewhere [8] because isolation of *Xenopus* oocytes using different preparations or batches of collagenase may alter the way they subsequently express mCAT-2 transport activity. Nevertheless, the data in Ref. [8] are clearly consistent with at least two components of transport for mCAT-2a expression (Fig. 2), although a single component of transport was assumed for purposes of analysis in Ref. [8]. Hence, expression of mCAT-2a, mCAT-1 and probably mCAT-2 in *Xenopus* oocytes can result in more than one kinetically distinguishable transport activity. Perhaps the mCAT proteins can exist in two kinetically distinguishable conformations or their transport characteristics might be altered by one or more accessory proteins expressed endogenously in *Xenopus* oocytes. It seems unlikely that exchange transport could somehow account for the curvature of the Hofstee plots in Figs. 1 and 2, since exchange of extracellular arginine for other cationic amino acids or for zwitterionic amino acids in association with Na^+ would be electrically neutral. Nevertheless, the currents we measured may not correspond directly to amino acid flux, since some uptake could have occurred as a result of electrically neutral exchange. Although it has been reported that more than one component of transport can be associated with expression of a putative accessory protein [4], this report is, to our knowledge, the first to show that multiple components of transport may be associated with expression of a putative transport protein.

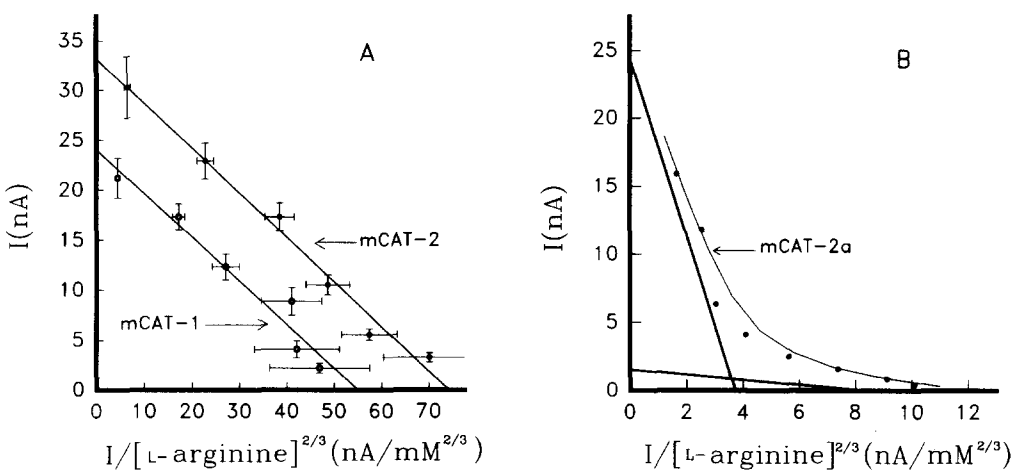

Fig. 3. Hofstee plot for L-arginine transport associated with mCAT-1 and -2 (A) or mCAT-2a (B) expression when the substrate concentrations are expressed as mmol per unit area rather than volume. Data for arginine transport in Figs. 1 and 2 were converted from volume-based to area-based concentrations by raising the concentrations to the 2/3 power. It is assumed that the concentration of arginine next to the membrane surface is the same or at least proportional to the concentration in the bulk solution and that it becomes unnecessary to consider a third dimension in calculating arginine concentration next to the membrane, once arginine molecules and the membrane have come in contact with each other. Although we know of no formal proof for this possibility, we also know of no such justification for the common although perhaps counter intuitive assumption that volume rather than surface area should be used in calculations concerning catalysis that occurs at a surface. Such considerations might be particularly important for cationic substrates like arginine, that might be oriented or restricted in their motion in two dimensions at the surface of biological membranes by negatively charged membrane phospholipids and the glycocalyx or by the membrane potential. In this scenario, the orientation of the cationic substrate at the membrane surface could also be the orientation of the substrate received by the transport protein. The data were assumed to be linear in A (correlation coefficients 0.98 and 0.99), whereas two components of transport were resolved for the data in B using the method of Spears et al. [15]. In this regard, it should be noted that the theoretical lines in Figs. 1 and 2 could not simply be converted to the theoretical lines in this figure by raising the substrate concentrations to the 2/3 power. Hence, it may become possible to use goodness of fit criteria to distinguish volume-based and area-based transport models kinetically. An intriguing possibility is that catalysis by some mCAT proteins might be most appropriately assessed in relation to substrate concentration in two dimensions, whereas three dimensions may be appropriate for others.

Alternatively, curved Hofstee plots for some transport proteins could conceivably result from expressing substrate concentrations erroneously as quantities per unit volume rather than per unit area. This situation might arise for example, if it sometimes becomes unnecessary to consider a third dimension in calculating substrate concentrations adjacent to a biological membrane, once substrate molecules and the membrane have come in contact with each other. When the data in Figs. 1 and 2 for mCAT-1, -2 and -2a expression are adjusted to make the concentrations reflect surface areas rather than volumes, the resultant Hofstee plot becomes linear for mCAT-1 and -2 but remains curved for MCAT-2a (Fig. 3). By this analysis, expression of mCAT-2a could be associated with more than one transport activity, whereas expression of mCAT-1 and -2 may be associated with only one. MCAT-2 and -2a differ only in a 43-amino acid residue segment of their putative 657-residue primary structures [6–8]. Hence, it is conceivable that mCAT-2a exists in two conformations, one that might resemble mCAT-2 and another with a higher K_m value.

It should become possible to determine whether any or all of the mCAT proteins can assume two kinetically distinguishable conformations when it becomes practicable to reconstitute the purified proteins and their activities in liposomes. Reconstitution of mCAT-associated transport activity may, however, require accessory proteins, or the mCAT proteins may themselves be accessory proteins rather than transporters. Regardless of whether any of these possibilities is correct, however, it is clear from the studies and analysis presented here that multiple components of transport may be associated with expression of a putative transport protein. In light of these findings, it seems important to reassess the transport kinetics associated with expression of other transport proteins since they too may catalyze or otherwise influence more than one kinetically distinguishable transport process.

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