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Importance of direct determination of amino acid co- and counter-transport stoichiometries

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Summary. The stoichiometry of amino acid transport with co- or counter-substrates of a given system has been found to vary with the amino acid species. This phenomenon has been studied directly in only a few cases, however, by measuring the fluxes of the substrates simultaneously. More frequently, the apparent transport stoichiometries of single amino acid species with co- or counter-substrates are estimated indirectly by thermodynamic criteria or cooperative kinetic effects. Unfortunately the latter indirect measures of apparent stoichiometry often yield different results than direct measurement of simultaneous fluxes. These differences often cannot be explained by uncoupled transport of one of the co- or counter-substrates or by other characteristics of the transport process that would make the direct measurement of stoichiometry inaccurate. For these reasons, investigators are encouraged to measure the stoichiometry of transport directly by measuring simultaneous fluxes of co- and counter-substrates. Indirect measures of apparent stoichiometry may, however, reflect important details of a transport mechanism even if they are inconsistent with the actual stoichiometry of transport.

Keywords: Amino acids – Kinetics – Thermodynamics – Transport – Transporters

I. Introduction

Amino acids are transported in many instances in association with a co- or counter-substrate. Inorganic ions usually serve as co-substrates, whereas other amino acids are frequently required for exchange. Nevertheless, antiport may involve exchange of an inorganic ion for an amino acid sometimes along with yet another ion. In addition, uncoupled transport of some co- or counter-substrates appears to contribute significantly to the total transport catalyzed by some amino acid transport systems, and some systems catalyze more than one transport process (reviewed by Van Winkle, 1999). Finally,

some transport systems appear to have different stoichiometries of co- or counter-transport for different amino acid species, and the stoichiometry of transport may be regulated for a given amino acid in physiologically important ways. We discuss here the significance of these observations to our understanding of amino acid transport, and we emphasize to investigators the need carefully to measure transport stoichiometry directly under pertinent physiological conditions.

II. Discrepancies among different measures of transport stoichiometry

Only in a few instances (e.g., system Gly in Table 1) does direct determination of the stoichiometry of simultaneous migration of amino acids and co- or counter-substrates match well the apparent stoichiometry estimated by kinetic or thermodynamic means. Indirect kinetic assessment of apparent transport stoichiometry involves determination of how one substrate promotes its own transport or that of a co- or counter-substrate (i.e., determination of Hill coefficients). Somewhat similarly, thermodynamic measures of apparent transport stoichiometry involve determination of the extent to which a gradient of one substrate may drive formation and maintenance of a gradient of a co- or counter-substrate. Of course, uncoupled migration of a substrate in addition to its coupled transport with another substrate would decrease its ability to form a gradient of the other substrate. Such uncoupled transport would lead to underestimation of transport stoichiometry by thermodynamic means. In contrast, this stoichiometry would be overestimated if the simultaneous migration of radiolabeled substrates were measured. Such uncoupled transport of inorganic ions has in fact been observed for some members of the sodium- and chloride-dependent, neurotransmitter transporter superfamily (reviewed by Palacín et al., 1998). Uncoupled transport of the neurotransmitters themselves has, however, not been observed for members of this transporter superfamily. Therefore, uncoupled transport cannot account for the discrepant estimates of transport stoichiometry for a member of this superfamily apparently expressed also in pigeon erythrocytes (i.e., system β in Table 1). Moreover, uncoupled transport has not been observed for most of the other transporter superfamilies listed in Table 1. Hence, uncoupling cannot in general account for the discrepant estimates of transport stoichiometry obtained by various means.

In the case of system β, actual co-migration of radiolabeled β-alanine and Na^+ occurs in a ratio of 1:1, whereas higher than first order kinetics are observed for the influence of the Na^+ concentration on β-alanine transport. Similarly, estimates of transport stoichiometry by various means give discrepant results for system X^-_{AG} (EAAT proteins) (Table 1). One might conclude from these data for system X^-_{AG} that transport of two Na^+ ions with each anionic amino acid molecule is the true stoichiometry of co-substrate migration, since this stoichiometry was determined by direct measurement of the simultaneous fluxes of Na^+ and glutamate. Unfortunately, such simultaneous flux measurements were not performed over the wide range of possible gluta-

Table 1. Amino acid transport systems for which the stoichiometry (coupling ratio) of co- or counter-transport has been measured directly and compared to indirect (thermodynamic or cooperative kinetic) estimates (see references for methods)

System	Probable transporter (accessory protein)	Transporter (accessory protein) Superfamily	Coupling ratio (co- or counter-substrates are shown in parentheses) as determined by measuring;			
			Thermodynamic effects	Cooperative kinetic effects	Simultaneous substrate fluxes	References[a]
ASC	ASC	ASC/EAAT	N.D.[b]	1.0 (Na^+/AÅ)	0.22 to 4.5[c]	1, 2
$b^{o,+}$	$b^{o,+}AT$[d] (BAT)	Na^+-independent (BAT and 4F2hc)	1.0 (AA^+/AÅ)	N.D.	1.0 to 30.0[c]	3
β	TAUT	Na^+- and Cl^--dependent	N.D.	2.0 (Na^+/β-AÅ)	1.0	1, 4
Gly	GLYT	Na^+- and Cl^--dependent	N.D.	>1.0 (Na^+/AÅ)	1.5	1
X^-_{AG}	EAAT3 (human)	ASC/EAAT	3.2 (Na^+/Glu^-) (determined using 5 to 300μM Glu^-)	2.3	N.D.	5
X^-_{AG}	EAAT3 (rabbit)	ASC/EAAT	N.D.	1.4[e] (1.0 mM Glu^-) 2.0 (0.2 mM Glu^-) ~10.0 (40μM Glu^-)	2.1 2.3 N.D.	6

[a] References: 1) Wheeler and Christensen, 1967; 2) Koser and Christensen, 1971; 3) Coady et al., 1996; 4) Ramamoorthy et al., 1994; 5) Zerangue and Kavanaugh, 1996; 6) Kanai et al., 1995.

[b] Abbreviations: *AÅ* zwitterionic α-amino acid; AA^+ cationic α-amino acid; *β-AÅ* zwitterionic β-amino acid; Glu^- glutamate anion; *N.D.* not determined.

[c] Range of values is for different amino acid substrates (see Table 2) and is not owing to variability for a given substrate.

[d] A system $b^{o,+}$ transporter ($b^{o,+}$AT or BAT1) was cloned recently by the International Cystinuria Consortium (human) and by Chairoungdua et al. (rat). The studies reported here are for expression of the BAT accessory protein in *Xenopus* oocytes which apparently express an endogenous $b^{o,+}$AT.

[e] Different values are obtained depending on the Glu^- concentration shown in parentheses. The values calculated in our reassessment of the original data and shown here are nearly the same as those reported by the authors, although the authors did not report a value for their data at 40μM Glu^-.

mate concentrations that may be present at various times in different portions of the central nervous system. Particularly when the glutamate concentration is lowered into the micromolar range, the kinetics of how glutamate transport depends on the Na^+ concentration change dramatically (Table 1). This large change in the value of the Hill coefficient probably overestimates the magnitude of the change in the transport stoichiometry proposed below. Nevertheless the change in the Hill coefficient value is consistent with the possibility that the transport stoichiometry also changes. As pointed out by Zerangue and Kavanaugh (1996), the co-transport of three Na^+ ions with each glutamate molecule might be needed sufficiently to reduce the extracellular glutamate concentration in the central nervous system to a non-excitatory level. We propose that some EAAT proteins may function at relatively high glutamate concentrations to take up greater quantities of glutamate by coupling its transport to only two Na^+ ions. As the glutamate concentration is lowered, however, the same proteins may be regulated to increase the stoichiometry of co-transport to, say, three Na^+ ions per glutamate molecule in order to accomplish glutamate uptake against a steeper gradient.

III. Importance of determining stoichiometry for various amino acid substrates of the same transport system

Another interesting aspect of the data presented in Table 1 is that the transport stoichiometry observed by direct measurement of substrate fluxes varies with the amino acid substrate species. In the case of system ASC, the measured stoichiometry varies from about 0.22 to 4.5 Na^+ ions per amino acid molecule (Table 2). Furthermore, the number of Na^+ ions transported with each amino acid molecule can be increased in the case of proline from 0.22 to about 3.2 through its *trans* hydroxylation (Table 2). Proline *trans* hydroxylation appears to increase the rate at which system ASC cycles by about 80-fold (Van Winkle, 1999). Moreover, this chemical modification of the amino acid substrate also seems to increase the probability that the Na^+ ion co-substrate will be displaced from the obligate ASC antiporter by another Na^+ ion on the other side of the membrane (Fig. 1). Consequently, system ASC transports *trans* hydroxyproline about six-times more rapidly than it transports proline (reviewed by Van Winkle, 1999). In a similar vein, α-carbon methylation of alanine appears dramatically to reduce the rate at which the resultant α-aminoisobutyrate is transported by system $b^{o,+}$ (Table 2), although the rate of concomitant arginine antiport is apparently unaffected by this change in structure of the zwitterionic counter-substrate (Coady et al., 1996).

As discussed above, one mechanism by which a structural change in a co- or counter-substrate could alter the stoichiometry of transport is by modifying the relative probability that each of the co- or counter-substrates will be displaced from the transporter in a given half cycle of transport. In the case of system ASC, a Hill coefficient of 1.0 is consistent with the possibility that one

Table 2. The stoichiometry of co- or counter-transport varies with the amino acid substrate species for each of the two systems for which the stoichiometries have been measured directly

System	Co- or counter-substrates	Amino acid substrate(s)	Coupling ratio (stoichiometry) Determined by measuring		
			Simultaneous substrate fluxes	Thermodynamic or kinetic effects	References[a]
ASC	Na^+/AÅ[b] (co-substrates)	L-cysteine	4.5	–	1, 2
		L-threonine	4.50 ± 0.12	–	
		L-serine	3.94 ± 0.13	–	
		L-4-*trans* hydroxyproline	3.16 ± 0.05	–	
		L-alanine	2.52 ± 0.08	1.0 (kinetic)	
		L-asparagine	1.66 ± 0.007	–	
		L-proline	0.22 ± 0.02	–	
$b^{o,+}$	AA^+/AÅ (counter-substrates)	Arg/Ala	1.0	1.0 (thermodynamic)	3
		Arg/AIB	30.0	1.0 (thermodynamic)	

[a] References: 1) Wheeler and Christensen, 1967; 2) Koser and Christensen, 1971; 3) Coady et al., 1996.
[b] Abbreviations: *AÅ* zwitterionic α-amino acid; AA^+ cationic α-amino acid; *AIB* α-aminoisobutyrate; *Ala* L-alanine; *Arg*, L-arginine.
[c] Standard errors were calculated from the standard deviations originally reported and as discussed elsewhere (Van Winkle, 1999).

Na^+ ion crosses the biomembrane with one amino acid molecule during each transport half cycle. Differences in the transport stoichiometry for different amino acid species could then arise from differences in the relative probabilities that one or the other of the co-substrates will be displaced by a pertinent substrate ion or molecule at each face of the biomembrane (e.g., Fig. 1).

IV. Conclusion

Investigators must remain open to the possibility that a system under investigation may transport different amino acids with their co- or counter-substrates in different stoichiometries depending on the structures of the amino acids. Moreover, the transport stoichiometry for a given amino acid may be regulated to increase the quantity of the amino acid transported or the gradient against which its net transport may occur. Although the results of thermodynamic and kinetic studies may be useful, it is important to measure transport stoichiometry directly by measuring the simultaneous fluxes of co- or counter substrates. While such fluxes have occasionally been measured (e.g., Koepsell et al., 1984; Kanai et al., 1995), they are not usually studied thoroughly under a variety of possible physiological conditions for most amino acid transport systems (Palacín et al., 1998; Van Winkle, 1999). Such studies for all transport systems are needed, however, in order to formu-

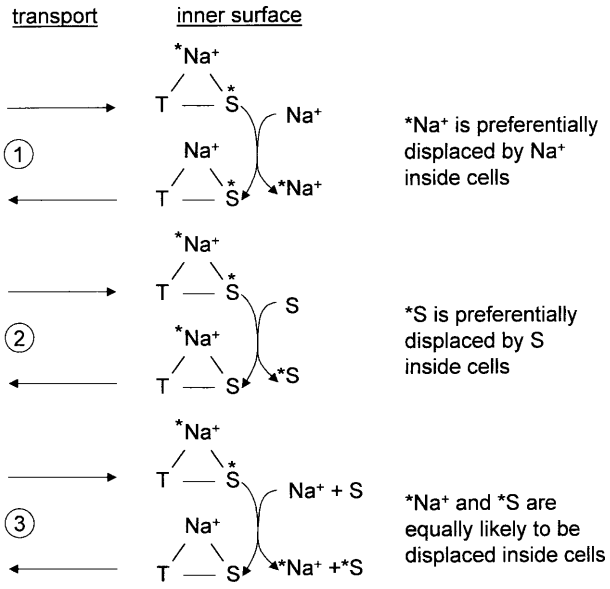

Fig. 1. Model to account for differences in the co-transport stoichiometry of Na^+ with various zwitterionic amino acids via system ASC. It is assumed that one Na^+ ion and one amino acid molecule actually traverse the biomembrane more or less together via system ASC in any one transport half cycle. The latter possibility is the simplest, and it is consistent with the observed Hill coefficient value of one for the dependence of alanine uptake on the Na^+ concentration (Table 2). Differences in the transport stoichiometries for different amino acid substrates are proposed to arise because the probabilities that the labeled amino acid molecule *S, or the labeled Na^+ ion, $*Na^+$, will be displaced from the transporter by unlabeled forms of these substrates on the other side of the membrane vary among amino acid species. Hence, the Na^+/amino acid transport stoichiometry exceeds one when $*Na^+$ is more likely to be displaced (case 1) and is less than one when *S is more likely to be displaced (case 2). This stoichiometry is one when *S and *Na are equally likely to be displaced (case 3). In the case of displacement of *S by S, *S and S may not be the same amino acid species. (Modified from Koser and Christensen, 1971)

late reasonable models for the mechanisms of transport and to determine whether transport stoichiometry may be regulated in physiologically important ways.

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