ENERGETICS OF AMINO ACID TRANSPORT

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While leucine absorption by human erythrocytes proceeds by facilitated diffusion, and analogous systems occur on one face of polar epithelial cells, the vertebrate systems of amino acid transport that have been studied most widely concentrate amino acids up to about 50-fold corresponding to a free energy increase of the order of 10 kJ mol\(^{-1}\). Much early work had as its main goal the characterisation of the number and specificity of the carrier systems involved, with the object of understanding both how solute concentration was effected at the expense of metabolic energy (the so-called energy coupling problem) and, further, how relatively hydrophilic or polar molecules traverse the hydrophobic barrier represented by the plasma membrane. In the broader biological context that includes both prokaryotes and eukaryotes, the systems that utilize ATP directly are to be distinguished from those based on redox reactions, on the one hand, and on the other hand from secondary transport systems that utilize an electrochemical gradient of either sodium ions or protons acting across the plasma membrane to drive a flow of organic solute either in the same direction (symport) or in the opposite direction (antiport). Only some of these diverse processes have as yet been studied in any detail (Eddy, 1990). In mammalian tissues, the Na\(^+\)-linked and proton-linked co-substrate systems probably provide the main mechanisms of amino acid and peptide accumulation. In particular the so-called \(A\) system of amino acid transport of mouse ascites tumour cells, that accepts many common neutral amino acids, including alanine, glycine and the metabolically inert analogues 2-aminoisobutyrate and 2-N-methylaminoisobutyrate, has been extensively investigated from the standpoint of its energetic and kinetic characteristics. Furthermore, the glycine and sarcosine carrier of the avian erythrocyte was used by Vidaver in the first formal demonstration of the ability of the sodium gradient in the absence of ATP to drive amino acid transport.

The Glutamyltranspeptidase Cycle

Meister has postulated that the membrane-bound enzyme \(\gamma\)-glutamyl-transpeptidase may participate directly in amino acid transport by donating \(\gamma\)-glutamyl residues from intracellular glutathione to incoming amino acids. The resultant \(\gamma\)-glutamylamino acid would break down into the free intracellular amino acid and 5-oxoproline that was subsequently reincorporated into glutathione (Meister & Anderson, 1983). The overall cycle of reactions is a costly one in energetic terms, consuming 3 ATP per mole of amino acid absorbed. While it is known that various tissues contain the relevant enzymes, the ability of the transpeptidase to transfer amino acid residues vectorially in the manner required by the hypothesis has not been demonstrated. Furthermore, a mutant human fibroblast cell line lacking the transpeptidase concentrated various amino acids in a similar fashion to its genetic parent (Pellefigue et al., 1976). Similarly, in the exocrine

*Mammalian Amino Acid Transport*, Edited by M.S. Kilberg and D. Häussinger, Plenum Press, New York, 1992
pancreas, marked inhibition of the enzyme by acivicin failed to inhibit uptake of either glutamine or alanine (Sastre et al., 1991). It seems clear that the primary mechanism of amino acid transport in these instances is not based on the γ-glutamylcycle. As Na+-dependent or proton-dependent symports have now been detected in many mammalian tissues, arguments in favour of a role for the transpeptidase in a second and parallel mechanism of amino acid accumulation need to be based on a careful assessment of the role of ionic gradients in the overall process.

Kinetic and Thermodynamic Models of the Co-substrate System

The possibility that an outflow of K+ ions might propel the uptake of glycine into mouse ascites tumour cells was first proposed by Christensen and his colleagues (antiport relationship). The mechanism was envisaged as an extension of the principle that membrane carriers, by virtue of the fact that their solute binding sites are alternately exposed either on one side or the other of the cell membrane, can couple an inflow of one solute A to a counterflow of solute B. In effect an initial gradient of A, acting across the cell membrane is converted to a transient gradient of B. Later work by Crane on glucose uptake in the intestine focussed attention on the further possibility that an inflow of Na+ ions might drive a parallel inflow of solute (symport relationship).

Equilibrium Carrier Models

Let the difference in electrochemical potential of Na+ on the two sides of the cell membrane be $\Delta \mu_{Na}$, $\Delta \mu_{K}$ and $\Delta \mu_{S}$ being the corresponding quantities for K+ and the solute, S, respectively. In general n Na+ ions might accompany each molecule of S through the carrier and where such a system comes to thermodynamic equilibrium

$$\Delta \mu_{S} + n\Delta \mu_{Na} = 0$$

(1)

Similarly, an antiport relationship with m K+ ions leads to the equation

$$\Delta \mu_{S} - m\Delta \mu_{K} = 0$$

(2)

These equations can readily be extended to examples where both Na+ and K+ or Cl- participate as co-substrate ions (Table 1). A useful alternative form of Equation 1 describes the amino acid accumulation ratio $[S]_i/[S]_o$ in terms of $[Na^+]_o/[Na^+]_i$ and the membrane potential ($\Delta \psi$)

$$[S]_i/[S]_o = ([Na^+]_o/[Na^+]_i)^n e^{-n\Delta \Phi/RT}$$

(3)

where $F$, $R$ and $T$ have their conventional meanings.

Table 1. Representative systems with Na+ and another cosubstrate ion.

<table>
<thead>
<tr>
<th>Potassium ions.</th>
<th>Aspartate uptake, chick intestine (Wingrove &amp; Kimmich, 1988); glial cells (Barbour et al., 1988); renal epithelia (Berteloot, 1986; Kinne et al., 1988).</th>
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<tr>
<td>Chloride and Potassium ions.</td>
<td>Serotonin uptake in platelets (Nelson &amp; Rudnick, 1982).</td>
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<tr>
<td>Chloride.</td>
<td>Glycine uptake, pigeon erythrocytes (Vidaver et al., 1976); γ-aminoisobutyrate uptake, brain (Keynan &amp; Kanner, 1988); the intestinal imino acid carrier and the intestinal β-alanine carrier (Munck &amp; Munck, 1990).</td>
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