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THE EFFICIENCY OF ENERGETIC COUPING BETWEEN Na^+ FLOW AND AMINO ACID TRANSPORT IN EHRLICH CELLS — A REVISED ASSESSMEN

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Summary

The overall efficiency of the coupling between transport of α-aminoisobutyrate and the entry of Na^+ in Ehrlich cells has previously been determined to be 8—10%. It was concluded that the efficiency is grossly inadequate to account for the energization of amino acid transport by the electrochemical potential gradient of Na^+, as postulated by the "gradient hypothesis". This conclusion had, however, not taken into account that a major part of the Na^+ entry is not coupled to α-aminoisobutyrate transport. The "intrinsic efficiency", which relates the amino acid transport to the coupled Na^+ entry only, has now been evaluated from available experimental data and found to be approximately adequate to account for the highest accumulation ratios for this amino acid reported. It is concluded that the gradient hypothesis cannot be rejected on energetic grounds.

According to the "gradient hypothesis" the active accumulation of neutral amino acids, notably those transported by the so-called A system, is completely energized by the electrochemical potential gradient of electrolyte ions. This view implies that the influx of amino acids is coupled to that of Na^+ (symport) and, possibly, to the efflux of K^+ (antiport). Though not denying that some energy of the mentioned gradients can be utilized for amino acid transport, other workers maintain that some, if not the major, part of the required energy stems directly from a metabolic reaction, via chemiosmotic coupling (primary active transport). The divergent views on this subject have been compiled [1].

The gradient hypothesis rests on two conditions, first, that the osmotic energy sources, i.e. the electrochemical potential gradients of Na^+ and K^+ are adequate, and second, that the coupling is tight enough to warrant the required efficiency of energy transfer. The first condition appears to be verified [2], at least if the sequestration of Na^+ in the nuclei is taken into account [3]. The second condition, however, has been doubted since in view of the small excess of energy available over that required, only an extraordinarily high efficiency of energy transfer would account for the transport work observed.

We recently attempted to assess the maximum coupling efficiency, η_{max}, quantitatively from experimental flux measurements, using the equations

$$\eta_{max} = \frac{q^2}{(1 + \sqrt{1 - q^2})^2} \qquad (1)$$

$$q^2 = \left(\frac{\partial J_A}{\partial J_{Na}}\right)_{X_A} \cdot \left(\frac{\partial J_{Na}}{\partial J_A}\right)_{X_{Na}} \qquad (2)$$

Since q was found to be about 0.5, and η_{max} accordingly 8%, it was concluded that this efficiency is far too low to account for the transfer of required energy between the Na^+ gradient and the amino acid transport [5].

Even though the value of η_{max} thus calculated appears to be correct, the conclusion drawn from it is probably not, as shown by the following analysis: η_{max} refers to the overall efficiency, expressed as

$$\eta = \frac{J_A X_A}{J_{Na} X_{Na}} \qquad (3)$$

in which J_A is the influx of α-aminoisobutyrate and J_{Na} the total passive influx of Na^+, which includes also fluxes along pathways not coupled to α-aminoisobutyrate transport (ΣJ^U_{Na}). X_A, X_{Na} are the driving forces resulting from the negative electrochemical potential differences of the amino A and of Na^+, respectively.

Accordingly $J_{Na} = J^C_{Na} + \sum J^U_{Na}$ (4)

These parts of the Na influx, ΣJ^U_{Na}, will depress the overall efficiency without being involved in α-aminoisobutyrate transport. As will be shown below, there is experimental evidence that ΣJ^U_{Na} may amount to more than half of overall J_{Na}. On the other hand, J_A in the above equation is the net influx of α-aminoisobutyrate which during accumulation is smaller than the pumping rate (J^C_A). J_A is even zero at static head. Consequently X_A in the above equation does not refer to static head, but to a lower value, namely to that at which the overall efficiency has reached its maximum.

In summary, the overall η_{max} of Eqn 2 is based on a J_{Na} which is too large, and on a J_A and a X_A which both are too small, so that η_{max} must be considerably lower than the intrinsic efficiency, η^{intr}, of the specific coupling under investigation. To assess η^{intr}, which can be expressed as

$$\eta^{\mathrm{intr}} = -\frac{J_A^C X_A}{J_{Na}^C X_{Na}} \tag{5}$$

we apply the principles of non-equilibrium thermodynamics. For the present problem we find it more convenient not to use the conventional, frictional notation but rather what we like to call the "quasichemical notation". It treats coupled processes like part of a chemical reaction with fixed stoichiometry and attributes deviations from stoichiometry to leakages [6] and other uncoupled pathways. If, for simplicity, we consider only the coupling between the fluxes of α-aminoisobutyrate, J_A, and the parallel one of Na^+, J_{Na}, the quasichemical equation could be written

$$\nu_A A_o + \nu_{Na} Na^+_o \rightleftharpoons \nu_A A_i + \nu_{Na} Na^+_i \tag{6}$$

and the overall reaction rate would be

$$J_r = L_r(-\sum \nu_i \Delta \mu_i) = L_r(\nu_A X_A + \nu_{Na} X_{Na}) \tag{7}$$

ν_i are the stoichiometric coefficients. L_r is the coefficient linking the overall reaction (J_r) to the total driving force ($-\Sigma \nu_i \Delta \mu_i$).
It follows that

$$\begin{aligned} J_A &= \nu_A J_r + \sum L_A^U X_A \\ &= (\nu_A^2 L_r + \Sigma L_A^U) X_A + \nu_A \nu_{Na} L_r X_{Na} \end{aligned} \tag{8a}$$

and

$$\begin{aligned} J_{Na} &= \nu_{Na} J_r + \sum L_{Na}^U X_{Na} \\ &= \nu_{Na} \nu_A L_r X_A + (\nu_{Na}^2 L_r + \sum L_{Na}^U) X_{Na} \end{aligned} \tag{8b}$$

The terms $\Sigma L_A^U X_A$ and $\Sigma L_{Na}^U X_{Na}$ stand for (ΣJ_A^U) and (ΣJ_{Na}^U), respectively, not pertaining to the above reaction, which we call leakage terms even though they may contain active transport terms, etc. The intrinsic efficiency of coupling at static head would then be:

$$\eta_o^{\mathrm{intr}} = -\left(\frac{\nu_A J_r X_A}{\nu_{Na} J_r X_{Na}}\right)_o = -\left(\frac{\nu_A X_A}{\nu_{Na} X_{Na}}\right)_o \tag{9}$$

It is obviously independent of the reaction rate, J_r, but contains the stoichiometric ratio ν_A/ν_{Na}, which for the present system is normally assumed to be one [7] but is at present not accurately determinable. In the present context, however, it would suffice to know the ratio $-(X_A/X_{Na})_o$, i.e. the intrinsic efficiency divided by ν_A/ν_{Na}, called by us the efficacy of accumulation, which can be determined experimentally. It can be derived from Eqn 8a by setting $J_A = 0$; so that

$$-\left(\frac{X_A}{X_{Na}}\right)_o = \frac{\nu_A \nu_{Na} L_r}{\nu_A^2 L_r + \Sigma L_A^U} \tag{10}$$

It would be equal to ν_A/ν_{Na} in the absence of leakage ($\eta^{intr} = 1$) but becomes smaller with increasing leakage. The right side can be determined independently of the left side, since according to Eqns 8a and 8b,

$$\frac{\nu_A\,\nu_{Na}\,L_r}{\nu_A^2\,L_r\;\Sigma L_A^U} = \left(\frac{\partial J_{Na}}{\partial J_A}\right)_{X_{Na}} \tag{11}$$

The value of $(\partial J_{Na}/\partial J_A)_{X_{Na}}$, i.e. the increment in Na^+ influx induced by an increment in α-aminoisobutyrate influx at constant electrochemical potential difference of Na^+, has been determined experimentally by us previously [5] and we found that at $[Na^+]$ = 70—130 mM in the extracellular fluid $(\partial J_{Na}/\partial J_A)_{X_{Na}}$ is about 0.6. Assuming that X_{Na} will not change much as long as $[Na^+]_f$ is constant and J_A not too large, it follows that the maximum efficacy of accumulation, to the extent that accumulation is energized by X_{Na} only, should not exceed 0.6. This is a value which comes close to the highest so far estimated. For example, the highest ratios of α-aminoisobutyrate accumulation in the literature are about 35 [2], corresponding to a X_A = —2000 cal/mole. Na^+ distribution ratios corrected for nuclear sequestration may be as low as 0.02 (Pietrzyk, C. and Heinz, E., unpublished), which together with an electrical potential difference of 25 mV [8] would give an X_{Na} of about 3000 cal/mole. Under these circumstances, $-(X_A/X_{Na})_o$ would be 0.67, not much higher than the value above. If the inverse K^+ gradient contributes to the driving force, the true $-(X_A/X_{Na})_o$ would be lower than 0.67. It would follow that the coupling between Na^+ influx and α-aminoisobutyrate transport appears to be tight enough, and the efficiency, whatever its true value may be, high enough to account for the highest α-aminoisobutyrate accumulation ratios found for Ehrlich cells.

That indeed a major part of Na^+ influx is not coupled to α-aminoisobutyrate transport can be supported by experimental evidence. It follows from Eqns 8a and 8b that

$$\left(\frac{\partial J_A}{\partial J_{Na}}\right)_{X_A} = \frac{\nu_A\,\nu_{Na}\,L_r}{\nu_{Na}^2 L_r + \Sigma L_{Na}^U} \tag{12}$$

By rearranging we obtain

$$\left(\frac{J_{Na}^U}{J_{Na}}\right)_{X_A = 0} = \frac{\Sigma L_{Na}^U}{\nu_{Na}^2\,L_r + \Sigma L_{Na}^U} = 1 - \frac{\nu_{Na}}{\nu_A}\left(\frac{\partial J_A}{\partial J_{Na}}\right)_{X_A} \tag{13}$$

which gives the fraction of uncoupled Na^+ influx within the total Na^+ flux to the extent that this flux is driven by X_{Na} only. Since the total Na^+ influx usually contains also the term $\nu_A\nu_{Na}L_rX_A$, Eqn 13 holds only if $X_A = 0$. Table I shows a few such values of the fraction of uncoupled Na^+ influx derived from our experimental results published previously [5].

The fraction of uncoupled Na^+ influx appears to decrease with increasing α-aminoisobutyrate concentrations, as could be expected. Since these

TABLE I

THE FRACTION OF Na^+ INFLUX NOT COUPLED TO α-AMINOISOBUTYRATE (AIB) TRANSPORT AT $X_A = 0$, (J^U_{Na}/J_{Na}) VALUES DERIVED FROM REF. 5.

$[AIB]_{ext}$ (mM)	$\left(\frac{\partial J_A}{\partial J_{Na}}\right)_{X_A}$	$(J^U_{Na}/J_{Na})_{X_A = 0}$, stoichiometric ratio	
		$\nu_{Na}/\nu_A = 1$	$\nu_{Na}/\nu_A = 2$
0.96	0.05	0.95	0.90
2.5	0.08	0.92	0.84
4.7	0.09	0.91	0.82
5.0	0.05	0.95	0.90
6.2	0.06	0.94	0.88
9.1	0.20	0.80	0.60
14	0.11	0.89	0.78
18	0.45	0.55	0.10

values hold for $X_A = 0$ only, they are too small if X_A is negative, i.e. if α-aminoisobutyrate is being accumulated, but they are too large if X_A is positive, i.e. if α-aminoisobutyrate flows downhill. Since the latter holds for our experimental data in the table the ratios may be considered as upper limits.

Eqn 13 shows also that the fraction of uncoupled Na^+ transport depends on the stoichiometric ratio ν_{Na}/ν_A. As has already been mentioned, ν_{Na}/ν_A cannot be accurately determined. However, it follows from Eqn 10 that an accumulation efficacy higher than 1 would indicate that $\nu_{Na}/\nu_A > 1$, because η^{intr} cannot exceed 1. For example, it had been found previously that $(\partial J_{Na}/\partial J_A)_{X_{Na}}$, and hence the efficacy of accumulation were higher with inhibited cells than for the controls, and in some cases seemed to exceed 1. Since η^{intr} is most probably smaller than 1, such high values for $(\partial J_{Na}/\partial J_A)_{X_{Na}}$ raise the suspicion that ν_{Na}/ν_A exceeds unity, i.e. that more than one sodium ion has to be moved for the coupled transport of one α-aminoisobutyrate molecule. The increase of $(\partial J_{Na}/\partial J_A)_{X_{Na}}$ due to metabolic inhibition had been taken to argue in favor of a direct coupling of α-aminoisobutyrate transport to a metabolic reaction. There seems to be no convincing way to explain this effect in terms of the gradient hypothesis, the more so since it is the uncoupled flux of α-aminoisobutyrate, not that of Na^+, which appears in Eqn 12.

Results similar to those in the table are obtained if the contribution of uncoupled Na^+ influx is estimated in a different way, namely by comparing the Na^+ influx at $J_A = 0$ with that at J_A^{max}. Also these values are upper limits since the Na^+ influx at $J_A = 0$ is likely to contain an exchange component whereas the extra Na^+ influx induced by α-aminoisobutyrate is a net movement, at least along the channel of Na^+–αaminoisobutyrate cotransport.

In conclusion, even though the maximum overall efficiency determined experimentally is extremely low, it can be shown with the same data that the small fraction of Na^+ influx involved in α-aminoisobutyrate transport is coupled tightly enough to this transport as to account for the accumulation actually observed. Hence it is difficult to reject the gradient hypothesis on energetic grounds.

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