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EVIDENCE AGAINST DIRECT COUPLING BETWEEN AMINO ACID TRANSPORT AND ATP HYDROLYSIS

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Summary

The degree of coupling (q) and the efficacy of accumulation $\left(-\frac{X_A}{A_{ch}}\right)_{J_A=0}$, i.e. the maximum static head accumulation ratio of α-aminoisobutyrate accounted for by ATP hydrolysis, have been assessed according to the rules of irreversible thermodynamics by comparing the mutual effect between α-aminoisobutyrate influx and ATP hydrolysis. Whereas the rate of α-aminoisobutyrate transport slightly rises with that of ATP hydrolysis, the latter was found to be completely independent of α-aminoisobutyrate transport. It was concluded that within the limits of experimental error, the degree of coupling and the efficacy of accumulation is negligible. In other words, there is no evidence that α-aminoisobutyrate transport in Ehrlich cells is directly energized by ATP hydrolysis.

It is still a controversial question whether the active transport of neutral amino acids into Ehrlich cells is energized entirely by the electrochemical gradient of Na^+ or whether it is, at least in part, directly coupled to an exergonic metabolic reaction, e.g. to the hydrolysis of ATP (primary active transport). Whereas a linkage between amino acid accumulation and Na^+ influx can hardly be debated, there is also evidence in favor of the second view: the same electrolyte gradients appear to drive amino acid transport about three times more powerfully with intact than with inhibited metabolism [1]. Furthermore, it could be shown that the amino acid α-aminoisobutyric acid may be transported uphill into the cell, even if the Na^+ and K^+ gradients are moderately inverted [2,3].

The energetic adequacy of the Na^+ gradient for amino acid transport has been assessed by determining the degree (q) of coupling between the two fluxes concerned [4]. It has been concluded from these results that the effi-

cacy of coupling is about 0.6, a value which is not very high but possibly sufficient (Heinz and Geck [8], following paper). The question now arises whether the coupling of amino acid to ATP hydrolysis is any better, tighter and more efficient than this to the extent it should be if the transport is primarily active.

Previously it had been observed that Ehrlich cells under normal conditions consume about 8 μmoles O_2 per g (dry wt) per min, and that this rate remains unchanged if the extracellular glycine concentration is increased from 1.7 to 16.5 mM, corresponding to an increment of net glycine uptake from 4.6 to 13.2 μmoles/g per min [5]. Obviously in these experiments the extra O_2 requirement for amino acid transport is so small compared to the basal O_2 consumption that it may remain within experimental error. Furthermore, a regulatory device (feedback) by which more ATP is being channeled into the transport process at the expense of other pathways cannot be excluded.

Hence, we endeavored to study the linkage between amino acid transport and ATP hydrolysis directly, and under conditions where resynthesis of ATP is either suppressed or curtailed at a rate similar to that of amino acid transport. The experiments have been designed and evaluated on the theoretical basis briefly outlined as follows:

In the "quasichemical notation" [6] of irreversible thermodynamics the basic reaction underlying chemiosmotic coupling can be written as [7]:

$$\nu_A A' + \nu_s S \rightleftharpoons \nu_A A'' + \nu_p P \tag{1}$$

A being the transported species, here the amino acid α-aminoisobutyrate, S and P being substrate and product, respectively, of the driving chemical reaction, here ATP hydrolysis. ν denotes the stoichiometric coefficient for each participant, and the superscripts ' and " refer to extracellular and intracellular, respectively. The rate of the overall reaction (J_r) would be

$$J_r = L_r(-\Sigma\nu_i \mu_i) = L_r \nu_A X_A + L_r \nu_{ch} A_{ch} \tag{2}$$

X_A is the conjugate driving force for A($= -\Delta\mu_A$), the negative difference in electrochemical potential difference. $A_{ch} = -(\nu_p \mu_s - \nu_s \mu_s)$, the affinity of the driving chemical reaction. ν_{ch} is probably unity unless the stoichiometry of Reaction 1 is defined as involving more (or less) than one ATP hydrolysed per transport cycle. L_r is the coefficient connecting the overall reaction rate (J_r) to its corresponding driving force ($-\Sigma\nu_i\mu_i$). $L^u{}_A$ and $L^u{}_{ch}$ are the corresponding coefficients referring to leakages or parallel pathways of α-aminoisobutyrate transport and ATP hydrolysis uncoupled with Reaction 1. Since there may be more than one of each, the Σ sign is used. It follows that

$$\begin{aligned} J_A &= \nu_A J_r + \Sigma L^u{}_A X_A \\ &= (\nu^2{}_A L_r + \Sigma L^u{}_A) X_A + \nu_A \nu_{ch} L_r A_{ch} \end{aligned} \tag{3}$$

$$\begin{aligned} J_{ch} &= \nu_{ch} J_r + \Sigma L^u{}_{ch} A_{ch} \\ &= (\nu^2{}_{ch} L_r + \Sigma L^u{}_{ch}) A_{ch} + \nu_A \nu_{ch} L_r X_A \end{aligned} \tag{4}$$

The degree of this coupling is

$$q = \left(\frac{\partial J_A}{\partial J_{ch}}\right)_{X_A} \cdot \left(\frac{\partial J_{ch}}{\partial J_A}\right)_{A_{ch}} \tag{5}$$

and the efficacy of accumulation at static head is obtained from Eqn 3 by setting $J_A = 0$;

$$-\left(\frac{X_A}{A_{ch}}\right)_{J_A = 0} = \left(\frac{\partial J_{ch}}{\partial J_A}\right)_{A_{ch}} = \frac{\nu_A \nu_{ch} L_r}{\nu^2_A L_r + \Sigma L^u_A} \tag{6}$$

Accordingly, only $\left(\frac{\partial J_{ch}}{\partial J_A}\right)_{A_{ch}}$ needs to be determined experimentally to estimate $-\left(\frac{X_A}{A_{ch}}\right)_{J_A = 0}$, the "efficacy of accumulation", and both $\left(\frac{\partial J_{ch}}{\partial J_A}\right)_{A_{ch}}$ and $\left(\frac{\partial J_A}{\partial J_{ch}}\right)_{X_A}$, to estimate q, the "degree of coupling".

A direct (chemiosmotic) coupling of α-aminoisobutyrate transport to ATP hydrolysis would be indicated if q were not only positive but greater than 0.5, the q previously found for the coupling between α-aminoisobutyrate transport and (passive) Na^+ influx. This is because the present q might reflect a stimulation of (Na^+—K^+)-ATPase owing to an increased flow of Na^+ into the cell associated with α-aminoisobutyrate uptake. In this case, q would refer to the overall coupling between two reactions in series, the α-aminoisobutyrate-induced Na^+ influx on the one hand and the Na^+-induced ATP splitting on the other. Since in this case

$$q_{overall} = q_1 \cdot q_2$$

q_1 and q_2 being the individual degrees of coupling of each reaction in series, $q_{overall}$ must be smaller than each individual q.

Experimentally, the intracellular ATP hydrolysis was first investigated at different rates of α-aminoisobutyrate transport; after resynthesis of ATP by oxidative phosphorylation had been stopped by oligomycin or antimycin A, the ATP disappearance inside the cells was followed with and without α-aminoisobutyrate in the medium.

In Fig.1 it is seen that the level of ATP, after 1 min delay, steadily decays, apparently leveling off towards a final baseline. The increase of extracellular Na^+ from 2 to 135 mM appears to accelerate this decay slightly, presumably by stimulating the Na^+—K^+-activated ATPase. On the other hand, the addition of α-aminoisobutyrate does not seem to produce any change in

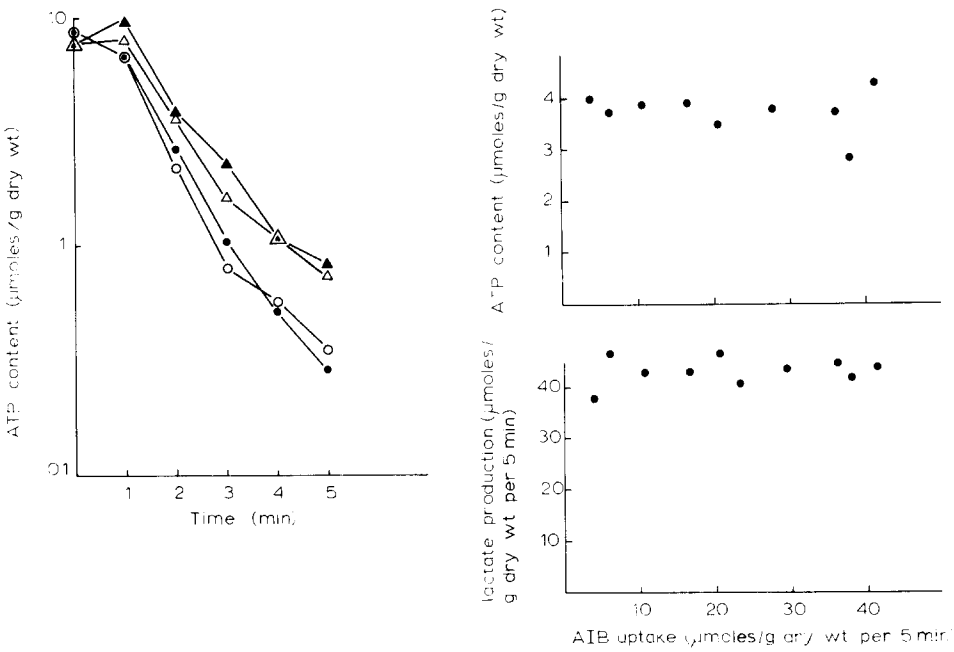

Fig. 1. Dependence of ATP hydrolysis on Na^+ and α-aminoisobutyrate. Abscissa: ATP content of cells in μmoles/g (dry wt), logarithmic notation. Circles, 135 mM Na^+; triangles, 2 mM Na^+.
Open symbols, without α-aminoisobutyrate;
solid symbols, with 10 mM α-aminoisobutyrate.
At time zero, the ATP level of the cells, as measured by the modified firefly method, was in all samples similar in the range between 8 and 9 μmoles/g (dry wt). Resynthesis of ATP was blocked by 6 μM oligomycin.

Fig. 2. Dependence of glycolytic ATP generation and turnover on α-aminoisobutyrate transport. Abscissa (both parts): Uptake of α-aminoisobutyrate (AIB) in μmoles/g (dry wt) per 5 min. Ordinate (upper part): ATP level of cells in μmoles/g (dry wt); (lower part): Rate of lactate formation (J_{lact}) in μmoles/g (dry wt) per 5 min. Oxidative phosphorylation was blocked by 6 μM oligomycin. Glycolytic ATP generation was maintained by 5 mM glucose, but kept down to a rate just sufficient to maintain the ATP content at a time-independent level by 2 mM phloretin. α-Aminoisobutyrate uptake was varied by the addition of α-aminoisobutyrate in the range of 0.1—10 mM. The ATP turnover is presumably identical with J_{lact} under these conditions, i.e. of similar magnitude as the maximum α-aminoisobutyrate uptake rate.

the rate of ATP decay. In the case of a reasonably efficient coupling between α-aminoisobutyrate transport and ATP hydrolysis, the latter, proceeding here at a rate similar to the former, should have been noticeably affected, even if several α-aminoisobutyrate molecules were transported per ATP molecule. Hence the result appears to suggest that $\left(\frac{\partial J_{ch}}{\partial J_A}\right)_{A_{ch}}$ is either zero or very small. To confirm this suggestion more conclusively, a more rigorous test has been carried out. Again, oxidative phosphorylation was blocked by oligomycin or antimycin A, and a moderate steady-state ATP level was main-

tained by glycolysis, whose rate was adjusted by partial inhibition with phloretin. The condition of constant A_{ch} was approximately fulfilled since, besides the level of ATP, also the levels of ADP and P_i did not change appreciably. Under these conditions, the rate of ATP turnover should be equivalent to that of lactate formation. Fig.2 shows that with and without α-aminoisobutyrate the steady-state ATP level remained about 4 μmoles/g (dry wt), and the glycolytic rate about 40 μmoles lactate per g (dry wt) per 5 min. If the ATP consumption were raised by the addition of α-aminoisobutyrate, the ATP level should drop, unless the extra ATP consumption were matched by a regulatory increase of the glycolytic rate. Neither an ATP drop, nor an increase in glycolysis, is observed, even though the rate of α-aminoisobutyrate uptake is increased stepwise from 0 up to 40 μmoles per g (dry wt) per 5 min. Hence $\left(\frac{\partial J_{ch}}{\partial J_A}\right)_{A_{ch}}$ is about 0, or so small that no coupling between amino acid transport and ATP hydrolysis can be detected.

The inverse relationship $\left(\frac{\partial J_A}{\partial J_{ch}}\right)_{X_A}$ was also tested in a similar manner, this time amino acid transport, J_A being plotted against ATP level and lactate production (J_{ch}); Fig.3 shows indeed a slight but significant increase in α-aminoisobutyrate transport if plotted versus lactate production, i.e. $\left(\frac{\partial J_A}{\partial J_{ch}}\right)_{X_A}$ appears to be different from zero.

However, as long as $\left(\frac{\partial J_{ch}}{\partial J_A}\right)_{A_{ch}}$ is zero, the degree and the intrinsic efficiency of coupling, as well as the efficacy of accumulation, $-\left(\frac{X_A}{A_{ch}}\right)_{J_A = 0}$, must be zero, too. In other words, the accumulation of α-aminoisobutyrate does not show a direct linkage to the ATP metabolism. Before a direct (chemiosmotic) coupling between ATP utilization and α-aminoisobutyrate transport is to be excluded, possible sources of error have to be considered. The dependence of ATP hydrolysis on α-aminoisobutyrate transport might, for instance, escape detection if either an excessive amount of ATP were utilized by processes other than α-aminoisobutyrate transport, or the stoichiometric ratio of coupling $\frac{\nu_A}{\nu_{ch}}$ were much higher than one. The first possibility is unlikely since in our experiments the ATP turnover was cut down to a rate similar to that of the α-aminoisobutyrate transport. The stoichiometric ratio,

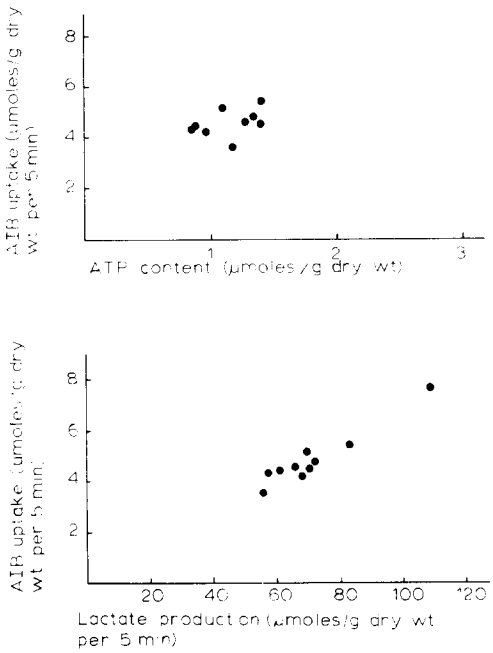

Fig.3. The dependence of α-aminoisobutyrate uptake on cellular ATP level and glycolytic ATP generation. Ordinates (both parts): uptake of α-aminoisobutyrate (AIB) μmoles/g (dry wt) per 5 min. Abscissa (upper part): level of cellular ATP in μmoles/g (dry wt); (lower part): rate of lactate production, in μmoles/g (dry wt) per 5 min. α-Aminoisobutyrate concentration was 0.1 mM in all samples. Glycolytic rate (J_{lact}) was varied by increasing inhibition with the addition of phloritin up to 2 mM. 5 mM glucose was added to maintain glycolysis. ATP concentration of each sample was measured at the end of the incubation period and correlated with the corresponding α-aminoisobutyrate transport.

$\frac{\nu_A}{\nu_{ch}}$, is not known but since about 10—12 kcal/mole are maximally available from ATP splitting and about 2 kcal are required for α-aminoisobutyrate transport, $\frac{\nu_A}{\nu_{ch}}$ cannot possibly exceed 5 or 6 for energetic reasons. Even with an a priori unlikely ratio of 5, an increase of α-aminoisobutyrate transport by about 40 μmoles/g (dry wt) per 5 min could be expected to raise J_{ch} by 8 μmoles/g per 5 min, or more, corresponding to 20% of J_{lact}. By contrast, the standard error of J_{lact} was only ± 5%. It follows that q is certainly less than 0.5, most likely even zero or negligible. Since, as mentioned before, this q would indicate a direct coupling with certainty only if it were greater than 0.5, the q of the coupling to Na^+ influx, a direct coupling between α-aminoisobutyrate transport and ATP hydrolysis, appears out of the question.

One may, on the other hand, wonder whether or not a slight coupling between α-aminoisobutyrate transport and ATP hydrolysis should be expected in any case, in view of the undoubted coupling between Na^+ extrusion and

ATP hydrolysis. Is it not obvious that if more Na^+ is swept into the cell owing to increased α-aminoisobutyrate transport, more Na^+ has to be pumped out in order that the right distribution of Na between cell and environment be maintained? While this question cannot yet be answered definitely, it is a priori clear that co transport between Na^+ and α-aminoisobutyrate could well increase without an appreciable change in Na^+ pumping rate. It is plausible that the more Na^+ is swept in by α-aminoisobutyrate, the higher the steady-state Na^+ level in the cytoplasm will become and the smaller the inward leakage of Na^+ will be. In other words, with increasing load of α-aminoisobutyrate the coupled Na^+ influx should increase at the expense of the uncoupled influx, even if the outward transport of Na^+ remains unchanged, for instance due to saturation of the pump. Under such conditions the α-aminoisobutyrate influx would show Michaelis—Menten kinetics, even if the number of transport carriers were unlimited. The maximum transport rate would then be equal to that of the Na^+ pump, i.e. to the supply of co-transportable Na^+.

Even though the full energization of α-aminoisobutyrate transport by Na^+, and possibly K^+, gradients is not yet proven under all conditions, a direct coupling of α-aminoisobutyrate transport to ATP hydrolysis appears to be still less likely in view of the present results. The question remains, however, what the energy source is in such cases in which the electrolyte gradients are clearly inadequate, for instance if Na^+ and K^+ gradients are inverted [3].

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