Hypothesis

THE DRIVING FORCE FOR PROTON(S) METABOLITES COTRANSPORT IN BACTERIAL CELLS

Hagai ROTTENBERG
Biochemistry Department, The George S. Wise Center for Life Sciences, Tel-Aviv University, Tel-Aviv, Israel

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1. Introduction

It is well established now that a large number of bacterial transport systems derive their energy from the proton electrochemical gradient, the protonmotive force, which is generated by proton pumps as was suggested by Mitchell [1–3].

$$\Delta p = \frac{\tilde{\mu}H}{F} = \Delta\Psi - ZpH \text{ where } Z = 2.3\ RT/F \qquad (1)$$

The mechanism suggested by Mitchell for these systems [1,4] is a coupling between the spontaneous uptake of protons and the non-spontaneous uptake of the substrate by a specific carrier. The mechanisms of these cotransport systems (symport) are the subject of this communication. Recently the protonmotive force was determined in various bacterial cells and cell membrane vesicles [5–10]. Although the findings differ considerably depending on the species, the type of vesicle and the method of determination, several conclusions can be drawn. In all the bacterial cells that were tested the proton pumps are directed outward. In all cases, the operation of the pump is associated with the formation of a membrane potential ($\Delta\Psi$) (inside negative). The proton gradient (ΔpH) however, is only large at a low external pH and decreases to very low values in neutral pH. In several systems such as *E. coli* [6] and *Rhodopseudomonas capsulata* (Rottenberg, in preparation) at pH above 7.5, ΔpH is even inverted and the cell becomes more acidic than the medium. Since $\Delta\Psi$ is essentially constant at pH range 5–9 the protonmotive force is reduced from high values at low pH to low values at high pH, where only $\Delta\Psi$ is significant.

Several specific models for proton(s)-substrate co-transport are presented below. These models take into account the reduction of ΔpH and Δp at high pH values, as discussed above, and the actual net charge which is carried by various substrates. These considerations require a transport system that could depend entirely on $\Delta\Psi$ at high pH whereas at low pH, ΔpH or both ΔpH and $\Delta\Psi$ can serve as the driving force. The suggested models allow the operation of the *same* carriers in two different modes at low and high pH.

In the construction of these models it was assumed that the *inward* moving complex that crosses the membrane is always neutral. The net charge of this complex is determined by the net charge of the substrate, the number of protons that are co-transported with the substrate and the charge of the carrier binding site which could be either neutral or negatively charged [11]. The mechanism of the transport cycle is envisaged as association of protons and substrate with the carrier binding site externally, the equilibration of the neutral complexed carrier site between the two membrane faces, dissociation of protons and substrate internally and outward movement of the free carrier site which could be facilitated by membrane potential if the carrier site is negatively charged (fig.1)*.

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*We do not suggest here that the carrier is a mobile carrier but only that the binding site moves across the membrane either by conformational changes or by rotation of the carrier.

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2. Co-transport systems for neutral substrates (fig.1A)

Sugars, neutral amino acids (Zwiterions) and other substrates that do not carry net charge at a physiological pH range are included in this category. According to our postulate the carrier-site-proton(s)-substrate complex must be neutral and therefore the charge on the carrier site itself must be negative and the valency equal to the number of protons carried with the substrate (fig.1a). A completely coupled co-transport system is driven by the sum of the electrochemical potential difference of the transported species and would come to equilibrium, i.e. net transport would vanish when:

$$\Delta\tilde{\mu}_A + n\Delta\tilde{\mu}_H = 0 \qquad (2)$$

where *n* is the stoichiometry of the cotransport system, nH$^+$/substrate. It should be noted that when net transport by the carrier vanishes *only* the coupled transport system is in equilibrium while each of the transported species need not be in equilibrium but could be in a steady state thus $\Delta\tilde{\mu}_A \neq 0$ and $\Delta\tilde{\mu}_H \neq 0$ but eqn. 2 hold. This is of course the state of interest for energy-dependent active substrate transport. Since the substrate itself is neutral we get from (1) and (2)

$$\mathrm{RTln}\,(\mathrm{A^oin/A^oout}) = -n(\mathrm{F}\Delta\Psi + \mathrm{RTln}\,(\mathrm{H^+in/A^+out})) \qquad (3)$$

or

$$\mathrm{Zlog}\,(\mathrm{A^oin/A^oout}) =$$

$$-n(\Delta\Psi - \mathrm{Z}\Delta\mathrm{pH}) = -n\Delta\mathrm{p} \qquad (4)$$

For $n = 1$, $\mathrm{Zlog}\,(\mathrm{A^oin/A^oout}) =$

$-\Delta\mathrm{p}$ and for $\mathrm{n} = 2$, $\mathrm{Zlog}\,(\mathrm{A\ in/A\ out}) = -2\Delta\mathrm{p}$

The value of Δp at low pH is sufficiently high to allow effective uptake with $n = 1$, while at high pH Δp is considerably reduced and it might be an advantage to have $n = 2$. The carrier can easily accomodate both mechanism if the p*K* value of the second proton site is at neutral pH, such a system can work with $n = 1$ at low pH and with $n = 2$ at high pH.

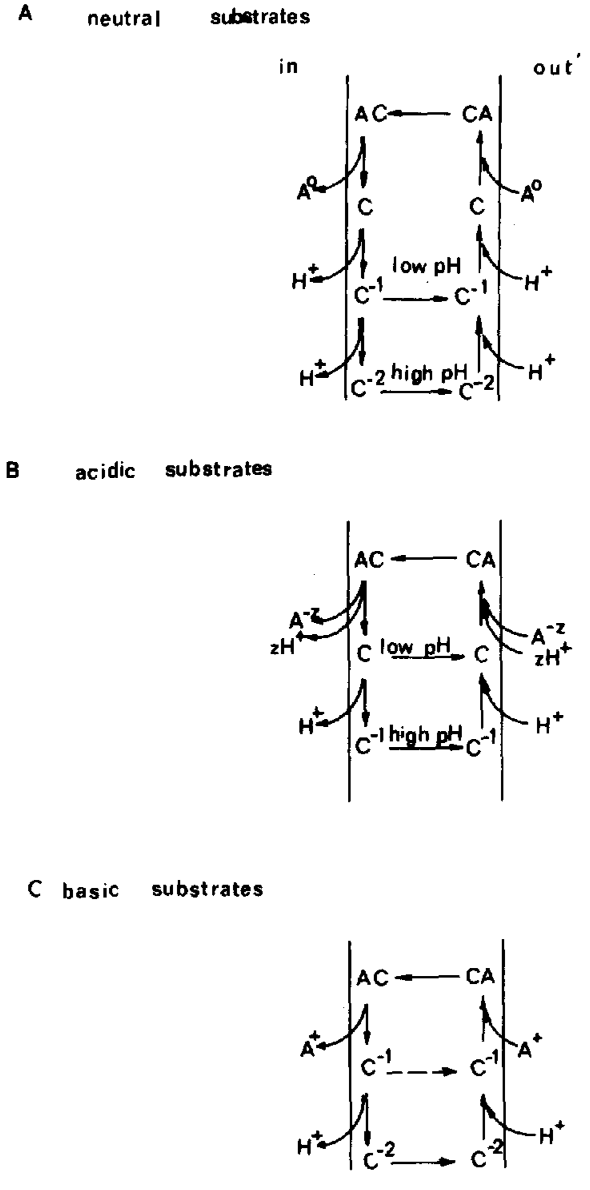

Fig.1. Models for co-transport carrier in bacterial cells.

3. Co-transport systems for negatively charged substrates (fig.1B)

Acidic substrates of intermediate metabolism, sugar-phosphates, acidic amino acids and other anionic substrates belong to this category. In this case electroneutrality does not require that the carrier would be negatively charged since balancing of the protons charge could be accomplished by the anionic substrate. When the transport vanishes, eqn. 2 determines the substrate distribution. However, in this case we get from eqn. 1 and eqn. 2 for substrate of charge $-z$.

$$-zF\Delta\Psi + RT\ln(A^{-z}in/A^{-z}out) =$$

$$-n(F\Delta\Psi + RT\ln(H^{+}in/H^{+}out)) \tag{5}$$

or

$$Z\log(A^{-z}in/A^{-z}out) = (z-n)\,\Delta\Psi + nZ\Delta pH \tag{6}$$

Thus, for neutral carriers, according to our phosphate $n=z$ and

$$\log(A^{-z}in/A^{-z}out) = z\Delta pH \tag{7}$$

and for negatively charged carriers $n = z + 1$ and

$$Z\log(A^{-z}in/A^{-z}out) =$$

$$-\Delta\Psi + nZ\Delta pH = -\Delta p + zZ\Delta pH \tag{8}$$

Thus at low pH when ΔpH is large one can expect some acidic substrate carrier to be neutral and the uptake to depend only on ΔpH. At high pH these carriers might dissociate (with neutral p*K*) and thus depend both on Δp and ΔpH according to eqn. 8. It is also possible that acidic substrate carrier would have low p*K* and be negatively charged at all pH values so that the substrate transport would always follow eqn. 8. It should be noted that the p*K* values of the substrates could also affect the driving force since the charge of the substrate might be different at low and high pH. For instance a substrate with 2p*K*s, one low and one at neutral pH transported by a carrier that is neutral at low pH and charged at high pH would obey the following relationship at low pH, from eqn. 7.

$$\log(A^{-1}in/A^{-1}out) = \Delta pH \tag{9}$$

and at high pH from eqn. 8

$$Z\log(A^{-2}in/A^{-2}out) = -\Delta\Psi + 2Z\Delta pH \tag{10}$$

4. Co-transport systems for positively charged substrates

Amine substrates and basic amino acids would belong in this category. In accord with our postulate these carriers must always be negatively charged with net charge which is at least equal to the substrate charge. However there is no obligatory coupling to proton transport since the membrane potential is sufficient to drive the transport against concentration gradients. For a carrier which is not coupled to proton transport, in equilibrium

$$\Delta\tilde{\mu}_{A^{+z}} = 0 \tag{11}$$

or

$$Z\log A^{+z}in/A^{+z}out = z\Delta\Psi \tag{12}$$

However because in low pH, ΔpH is quite large it is reasonable to assume that some of these transport systems would be also coupled to proton transport (fig.1c). Thus from eqn. 1 and 2

$$zT\Delta\Psi + RT\ln(A^{+z}in/A^{+z}out) =$$

$$-n\,(F\Delta\Psi + RT\ln H^{+}in/H^{+}out) \tag{13}$$

or

$$Z\log(A^{+z}in/A^{+z}out) =$$

$$-(n+z)\Delta\Psi + nZ\Delta pH = -n\Delta p - z\Delta\Psi \tag{14}$$

Therefore these systems are expected to depend mostly on ΔΨ and to a lesser extent on ΔpH. Table 1 summarizes the various possibilities of co-transport systems that conform to our basic postulate and the various forms of their driving force ($\Delta\tilde{\mu}_A + n\Delta\tilde{\mu}_H$).

5. The dependence of the rate of the co-transport on the driving force

Carrier kinetics in general is quite complex and presently there is not a single entirely satisfactory kinetic model which is compatible with all the data available [12]. It is therefore futile to attempt an

Table 1
Stoichiometry and driving force for metabolites co-transport systems suggested in this communication

Substrate charge	Carrier charge	Stoichiometry H^+/substrate	Driving force (mv)	Equation
A^0	C^{-1}	1	$Z\log(A^0\text{in}/A^0\text{out}) + \Delta\Psi - Z\Delta pH$	(4)
	C^{-2}	2	$Z\log(A^0\text{in}/A^0\text{out}) + 2\ (\Delta\Psi - Z\Delta pH)$	(4)
A^-	C^0	1	$Z\log(A^-\text{in}/A^-\text{out}) - Z\Delta pH$	(7)
	C^{-1}	2	$Z\log(A^-\text{in}/A^-\text{out}) + \Delta\Psi - 2Z\Delta pH$	(8)
A^{-2}	C^0	2	$Z\log(A^{-2}\text{in}/A^{-2}\text{out}) - 2Z\Delta pH$	(7)
	C^{-1}	3	$Z\log(A^{-2}/A^{-2}\text{out}) + \Delta\Psi - 3Z\Delta pH$	(8)
A^+	C^0	–	$Z\log(A^+\text{in}/A^+\text{out}) + \Delta\Psi$	(12)
	C^{-1}	1	$Z\log(A^+\text{in}/A^+\text{out}) + 2\Delta\Psi - \Delta ZpH$	(14)

$Z = 2.3\ RT/F$

exact treatment of the relationship between the rate of the co-transport carrier and their driving force. Nevertheless an approximate relationship that should be valid under specified *restricted* conditions can be derived. The most widely used equation for carrier transport is of the following form [13].

$$J_{net} = J_m\left(\frac{A\text{ out}}{K_t + A\text{ out}} - \frac{A\text{ in}}{K_t + A\text{ in}}\right) \qquad (15)$$

where J_m is the maximal rate of transport and K_t is an experimental constant. Let us denote $X = A/K_t$, then,

$$J_{net} = J_m\left(\frac{X\text{ out}}{X\text{ out} + 1} - \frac{X\text{ in}}{X\text{ in} + 1}\right) \qquad (16)$$

for X values not too different from one since

$\frac{X}{X+1} = \frac{1}{2} + \frac{1}{4}\ln X$ [14] we get,

$$J_{net} = \frac{J_m}{4}(\ln X\text{ out} - \ln X\text{ in}) = \frac{J_m}{4}\ln(A\text{out}/A\text{ in}) \qquad (17)$$

and since $\Delta\mu_A = RT\ln(A\text{ out}/A\text{ in})$, we get,

$$J_{net} = \frac{J_m}{4RT}\Delta\mu_A \qquad (18)$$

That is, when the substrate concentration is of the same order as the K_t, the rate of transport is a linear function of the chemical potential difference. For ions, $\Delta\tilde{\mu}_i$, the electrochemical potential difference must be considered, and since the electrical gradient and the chemical gradient are indistinguishable we may generalize,

$$J_{net} = \frac{J_m}{4RT}\Delta\tilde{\mu}_A \qquad (19)$$

In a co-transport carrier both substrates and protons are transported. However, if we assume that the binding or dissociation of the substrates is not rate limiting, we can regard the transported substrate as the combined substrate-proton(s) complex, AHn and $\Delta\tilde{\mu}_{AHn}$ is given by

$$\Delta\tilde{\mu}_{AHn} = \Delta\tilde{\mu}_A + n\Delta\tilde{\mu}_H \qquad (20)$$

we substitute eqn. 20 in eqn. 19 and get,

$$J_{net} = \frac{J_m}{4RT}(\Delta\tilde{\mu}_A + n\Delta\mu_H) \qquad (21)$$

In order to test this relationship it is best to try to measure the rate of uptake not initially but at a point where A in = A out = K_t. In these conditions eqn. 21 is expected to be reasonably accurate and since $\log(A\text{ in}/A\text{ out}) = 0$ the rate should be proportional to ΔpH and $\Delta\Psi$ components of the driving force as enumerated in table 1. Although there are very few published experiments that allow the testing of these models, work in progress in several laboratories should provide these data in the near future. The work of Kaback's group [3,8,11] indicates that for most of the respiration dependent transport systems, $\Delta\Psi$ might be sufficient for driving the co-transport since the uptake against concentration gradient is quite significant at high pH. Also, the work of this group indicate that the lactose carrier is probably negatively charged [11].

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