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Product Inhibition during Ion : Solute Cotransport Is an Alternative to Leaks as a Cause of Low Accumulations

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Summary. Ion : solute cotransporters frequency are incapable of achieving equilibrium between the solute accumulation and the transmembrane difference of the electrochemical potential of the ion. The presence of uncoupled flows of ion and solutes (leaks) is often advanced as an explanation. Here an alternative is discussed. The net accumulation of solute may be so slow that equilibrium can never be attained at finite times (e.g., several hours). Cotransporters may exhibit strong product inhibition, and the net influx of solute approaches zero far from equilibrium. The inherent slowness of net transport under these conditions is termed catalytic inefficiency. The likelihood that galactoside : H^+ cotransport in *Escherichia coli*, hexose : H^+ cotransport in *Chlorella vulgaris*, and D-glucose : Na^+ cotransport in brush-border membranes exhibit catalytic inefficiency is examined. The existence of strong product inhibition complicates the determination of the stoichiometry of cotransport and the characterization of chemically modified or mutant cotransporters.

Key Words cotransport · accumulation · nonequilibrium state · leaks · product inhibition

Introduction

The active transport of many nutrients is mediated by ion : solute cotransport (Mitchell, 1973; Crane, 1977). The driving free energy for the accumulation is provided by the action of primary ion pumps, which create transmembrane differences of the electrochemical potential of ions

$$\Delta\tilde{\mu}_{ION} = F \cdot \Delta\psi - RT \ln \frac{[ION']}{[ION'']} \qquad (1)$$

where F is 96, 490 A · sec · mol^{-1}, $\Delta\psi$ is the electrical potential difference, R is 8.314 J · mol^{-1} · K^{-1}, T is the absolute temperature, and [ION] is the concentration of a given ion in the outer (′) or inner (″) compartment. In their simplest form, cotransporters can be viewed as membrane proteins with binding sites for solute and ions, and having access to two major conformation states in which these binding sites can be exposed to two compartments (Fig. 1).

The goal of kinetic and thermodynamic investigations of cotransport is to explain two types of behavior: First, under certain conditions tight coupling of ion and solute fluxes can be demonstrated. The accumulation of solute comes into equilibrium with the driving free energy, for example $\Delta\tilde{\mu}_{H^+}$ in the case of a H^+ : solute cotransporter, according to

$$\frac{[S'']}{[S']} = \exp(-n_{H^+}\Delta\tilde{\mu}_{H^+}/RT) = \exp(\Delta\mu_S/RT) \qquad (2)$$

where n_{H^+} is the stoichiometry of cotransport, corresponding to the number of binding sites for the transported ions, and $\Delta\mu_S$ is the transmembrane difference in the chemical potential of the solute. Thus, according to Eq. (2), the accumulation ratio of solute due to H^+ : solute cotransport depends only on the driving free energy $\Delta\tilde{\mu}_{H^+}$ and the stoichiometry, but not upon the number of cotransporters in the membrane, the turnover number of the cotransporter k_{cat}, or the external concentration of solute $[S']$.

Second, the accumulation of solute in the apparent steady state is frequently observed to be lower than that predicted by Eq. (2) and to follow the relation

$$\frac{[S'']}{[S']} = \frac{[S''_{max}]}{[S'] + K_{ss}} \qquad (3)$$

(*cf.* Rickenberg et al., 1956), where S''_{max} is the maximal concentration of solute attainable internally and K_{ss} is a half-saturation constant usually similar to the half-saturation constant for active transport K_T. For example, at an external concentration of 1 mM, galactoside accumulation in *E. coli* can be as low as 5% of the prediction of Eq. (2) for $n_{H^+} = 1$ (Ahmed

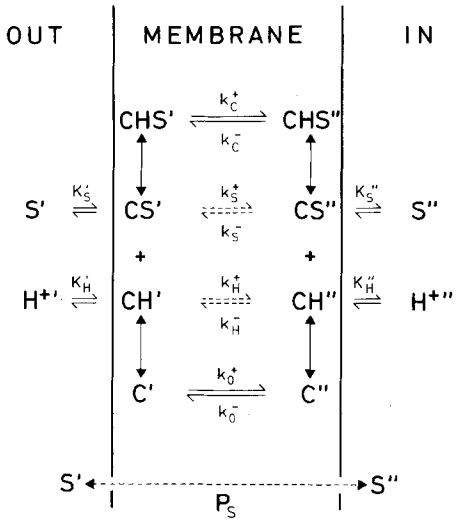

Fig. 1. Transmembrane fluxes of H^+ and a neutral solute S. Strictly coupled fluxes are mediated by an ion : solute cotransporter C following the paths indicated by solid arrows. Here random binding of the cosubstrates is assumed. Inner leaks (*cf.* Eddy, 1978) occur if the cotransporter mediates the movement of H^+ or S alone (dotted arrows k_H and k_S). The passive diffusion of the solute (dotted arrow P_S) represents an outer leak and is not mediated by a transporter

& Booth, 1981; Wright, 1986*b*). Thus, a complete elucidation of the mechanism of secondary active transport requires understanding the behavior described by Eqs. (2) and (3). Furthermore, the phenotypes of cells carrying mutant cotransporters and the accumulation of solute via chemically modified cotransporters are frequently tested under conditions where Eq. (3) and not Eq. (2) obtains. Thus, understanding how the behavior described by Eq. (3) arises is necessary to interpret such experiments correctly (*cf.* Neuhaus & Wright, 1983; Wright & Seckler, 1985). The presence of uncoupled flows of ion or solute across the membrane (dashed lines in Fig. 1) is usually advanced as an explanation for low accumulation (*cf.* Eddy, 1978). However, there is an unattractive feature of such explanations for low accumulations. In the steady state, characterized by $d[S'']/dt = 0$, there is a constant dissipation of $\Delta\tilde{\mu}_{ION}$ because $d[H^{+''}]/dt$ is not zero, and energy is wasted.

The purpose of this investigation is (*i*) to propose an explanation for low accumulations not relying on the uncoupled flows of cosubstrates but rather based upon the innate slowness (i.e., catalytic inefficiency) of the *net* transport of solute under some conditions, (*ii*) to demonstrate that this behavior leads to a relation similar to Eq. (3), (*iii*) to illustrate this with experimental data obtained for H^+ : galactoside cotransport in *E. coli*, and (*iv*) to suggest that catalytic inefficiency may be a widespread feature of cotransport. The presence of catalytic inefficiency during ion : solute cotransport could preclude ready estimates of stoichiometry and of the tightness of flux coupling from measurements of solute accumulation in the apparent steady state as well as an unambiguous interpretation of the phenotypes of mutants.

Results

The Need for an Alternative to Leaks

The galactoside : H^+ cotransporter of *E. coli*, product of the *lac*Y gene, can catalyze the accumulation of sugar up to levels several thousandfold above that in the external medium. Under many, but not all conditions, the stoichiometry of cotransport is near unity (e.g., West, 1970). Several observations suggest, however, that galactoside : H^+ cotransport is not always properly described by the thermodynamic statement of Eq. (2). First, the accumulation ratio decreases as the external concentration of galactoside $[S']$ increases (e.g., Rickenberg et al., 1956). In the case of lactose (Ahmed & Booth, 1981) and β-D-galactosyl 1-β-D-thiogalactoside (GalSGal[1]) (Wright, 1986*b*), a decrease in $\Delta\tilde{\mu}_{H^+}$ at higher galactoside concentrations could be eliminated as an explanation for this behavior. Second, the accumulation depends on the number of cotransporters in the membrane (Maloney & Wilson, 1973; Teather et al., 1980). Third, the accumulation depends on the turnover number k_{cat} for cotransport (Wright & Seckler, 1985). Fourth, the accumulation sometimes depends not simply on the magnitude but on the composition of $\Delta\tilde{\mu}_{H^+}$ (Ahmed & Booth, 1981).

An additional postulate could explain these observations. The fluxes of ion and solute across the membrane may not be strictly coupled (Eddy, 1978). Three leak pathways are represented by dashed lines in Fig. 1. One possibility is that the solute diffuses passively out of the cell (P_S). Rickenberg et al. (1956) interpreted the variation of the internal level of galactoside in the apparent steady state with the external concentration as the balance of saturable influx and nonsaturable efflux

$$\frac{C_T \cdot k_{cat} \cdot [S']}{[S'] + K_T} = P_S \cdot [S''] \qquad (4)$$

[1] GalSGal: β-D-galactosyl 1-thio-β-D-galactoside.

yielding

$$[S''_{max}] = \frac{C_T \cdot k_{cat}}{P_S} \tag{5}$$

where C_T is the level of cotransporter and k_{cat} the turnover number for active transport. For *E. coli* strain ML308-225 with 0.2 nmol galactoside : H^+ cotransporter/mg membrane protein, C_T amounts to 6 pmol/μl cytoplasm.

Two observations suggest this interpretation is incorrect. First, for hydrophilic disaccharides like lactose (Booth & Hamilton, 1980) or GalSGal (Wright et al., 1985; Wright, 1986*b*), the passive permeability of the membrane for these sugars is too small by a factor of 40 to explain the magnitude of the deviation from Eq. (2). Second, the left-hand side of Eq. (4) is inappropriate for an ion : solute cotransporter. The proper expression for the net flow of solute through the cotransporter is

$$\frac{d[S'']}{dt} = \frac{C_T(k_c^+\alpha'\beta'k_o^- - k_c^-\alpha''\beta''k_o^+)}{(1 + \alpha' + \beta' + \alpha'\beta')(k_c^-\alpha''\beta'' + k_o^-) + (1 + \alpha'' + \beta'' + \alpha''\beta'')(k_c^+\alpha'\beta' + k_o^+)} \tag{6a}$$

where $\alpha = [S]/K_S$ and $\beta = [H^+]/K_H$. The equation can be written for later use as

$$\frac{d[S'']}{dt} = \frac{C_T(B_1\alpha'' + B_2)}{A_1\alpha'' + A_2}. \tag{6b}$$

Equation (6) explicitly recognizes that the influx of galactoside depends not only on $[S']$ but also on $[H^{+\prime\prime}]$ and $[S'']$. Even if the outer leak P_S is the major pathway for galactoside efflux, the internal concentration of galactoside is not given by an equation of the form of Eq. (3), but rather by

$$A_1(\alpha'')^2 + A_2\alpha'' - \frac{C_T \cdot B_2}{P_S \cdot K''_S} = 0. \tag{7}$$

If A_1 were small, Eq. (7) could be simplified to the form of Eq. (3). A condition leading to a small value of A_1 would be $k_o^- \gg k_c^-\,[H^{+\prime\prime}]/K''_H$. This condition is not fulfilled in the case of the galactoside : H^+ cotransporter as substitution of the appropriate values discloses. Therefore, both quantitative and theoretical considerations suggest that the efflux of galactoside by passive diffusion cannot explain the uptake described by Eq. (3).

A second possibility is that the cotransporter can also catalyze the movement of H^+ or solute alone. This is called inner leak or slip and is represented by the dashed lines and the rate constants k_H and k_S in Fig. 1. In this case, the accumulation of solute attains a nonequilibrium steady state (i.e., $d[S'']/dt = 0$; $d[H^{+\prime\prime}]/dt \neq 0$) also, given by

$$\frac{[S'']}{[S']} = \frac{K''_S}{K'_S} \cdot \frac{k_c^+\beta' + k_s^+}{k_c^-\beta'' + k_s^-} \cdot \frac{k_o^- + k_H^-\beta''}{k_o^+ + k_H^+\beta'}. \tag{8}$$

Inspection of Eq. (8) reveals that inner leaks cannot explain low accumulations of galactosides for two reasons. The accumulation is not a function of either the level of cotransporter C_T or the external concentration of galactoside $[S']$, as experimentally observed.

The thermodynamic efficiency of a cotransporter can be quantitatively defined as

$$E_T = \lim_{t\to\infty} \frac{RT \ln([S'']/[S'])}{-n_{H^+} \cdot \Delta\bar{\mu}_{H^+}} \tag{9}$$

where the accumulation ratio is that measured at infinite time. E_T is unity when solute and ion fluxes are strictly coupled. In the following section, an explanation for low accumulation of solute in the absence of leaks ($E_T = 1$) is advanced.

Slow Rates of Net Transport Mimic the Effects of Leaks

The kinetic mechanism of the accumulation of GalSGal by the galactoside : H^+ cotransporter is known (Overath & Wright, 1983; Wright 1986*b*). In the absence of $\Delta\bar{\mu}_{H^+}$ the cotransporter behaves like a classical symmetrical carrier:

$k_c^+ \approx k_c^- \approx k_o^+ \approx k_o^- \approx 30\ sec^{-1}$, $K'_S = K''_S = 78\ \mu M$; $K'_H = K''_H = 50\ pM$.

The imposition of $\Delta\bar{\mu}_{H^+}$, changes k_c^+, k_o^+, and k_o^-; the imposition of ΔpH causes redistributions of the cotransporters by mass action. Therefore, the time course of GalSGal active transport can be calculated by integrating Eq. (6), yielding

$$\frac{A_1}{B_1}\alpha''(t) + \frac{A_2B_1 - A_1B_2}{B_1^2} \ln\left(\frac{B_1\alpha''(t) + B_2}{B_2}\right) = \frac{C_T \cdot t}{K''_S} \tag{10}$$

where $\alpha''(t)$ is the value of $[S'']/K''_S$ at time t. The value of $[S'']$ in the limit of *infinite* time for Eq. (10) is always that predicted by Eq. (2) because Eq. (10) was derived for $P_S = k_S^+ = k_S^- = k_H^+ = k_H^- = 0$ (i.e., no leaks).

The time courses of GalSGal uptake at several constant external concentrations between $[S'] =$

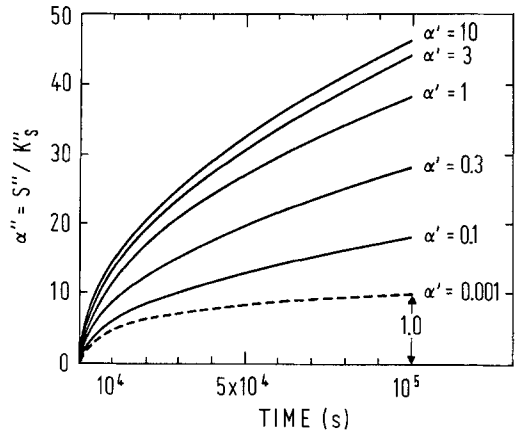

Fig. 2. Slow approach to equilibrium by a strictly coupled ion : solute cotransporter. The time course of the accumulation of galactoside is calculated for various constant external concentrations of galactoside ($\alpha' = [S']/K'_S = 0.001$ to 10, curves identified on the right; note expanded scale for $\alpha' = 0.001$). In the absence of $\Delta\tilde{\mu}_{H^+}$, $k_c^+ = k_c^- = k_o^+ = k_o^- = 30$ sec^{-1}; $K'_S = K''_S = 78$ μM; $K'_H = K''_H = 3$ nM (i.e., $pK_H = 8.5$; lowest of available estimates of the pK of the cotransported H^+ by Bentaboulet and Kepes (1981)). For *E. coli* ML308-225, C_T amounts to 0.006 nmol/μl cytoplasm. A value of $\Delta\tilde{\mu}_{H^+}$ is imposed ($\Delta\psi = -118$ mV, $\Delta pH = 1$) leading to a 1000-fold accumulation of galactoside in the limit of infinite time. Curves are calculated using values of k_o^- = 300 sec^{-1} and $k_o^+ = 3$ sec^{-1} to reflect the effect of the imposed $\Delta\psi$; ΔpH evokes active transport by mass action, and no rate or dissociation constants are altered (Overath & Wright, 1983; Wright et al., 1985; Wright et al., 1986). At finite times, the accumulation of galactoside lies below the equilibrium value (Eq. (2)). Note that in spite of a 10^4-fold variation in the external concentration of solute, the internal level varies only by a factor of 40 at 5×10^4 sec

0.078 μM ($\alpha' = 0.001$) and 780 μM are computed for $\Delta\psi = -118$ mV and $\Delta pH = 1$, corresponding to a 1000-fold accumulation at equilibrium (Fig. 2).

At a low external concentration, $[S'] = K'_S/1000$ ($\alpha' = 0.001$, dashed line in Fig. 2; note expanded scale), the accumulation of GalSGal comes into equilibrium with $\Delta\tilde{\mu}_{H^+}$ ($[S''] = 1000 \times [S']$; $\alpha'' = 1$). However, as the external concentration of GalSGal increases, progressively longer times are required to reach the equilibrium of Eq. (2). For example, when $[S'] = K'_S$ ($\alpha' = 1$), the value of $[S'']$ at equilibrium is $1000 \times S'$ ($\alpha'' = 1000$). After 10^5 sec (about 28 hr), the internal concentration of GalSGal is calculated to have attained only 4% of the equilibrium value. Importantly, similar curves are obtained if the parameters are varied over wide ranges. Therefore, the behavior observed in Fig. 2 can be a general property of cotransporters of similar design (*cf.* discussion in Wright et al., 1985; Wright, Seckler & Overath, 1986). Thus, although the fluxes of ion and solute are strictly coupled, the slowness of net transport (i.e., influx minus efflux) leads to submaximal accumulations at finite times and mimics the effects of leaks. The following analysis demonstrates that strong product inhibition, termed catalytic inefficiency, during cotransport is responsible for low accumulations.

Behavior of Cotransporters Far from Equilibrium

Inspection of the calculated internal levels of solute at a given time such as 5×10^4 sec in Fig. 2 reveals two points (*cf.* Eq. (3)). First, the internal level appears to approach a maximum with increasing external concentrations of galactoside (e.g., note how the curves converge with increasing α' at 5×10^4 sec). Second, this effect is more pronounced as the external concentration rises above K'_S ($\alpha' \geq 1$). The experimental value for the half-saturation constant K_T for the active transport of GalSGal corresponds to about $\alpha' = 0.5$. Thus, qualitatively at any given time, the accumulation appears to follow Eq. (3). This impression can be confirmed mathematically. The argument of the logarithm in Eq. (10) is a measure of the closeness to the equilibrium of Eq. (2)

$$\frac{B_1\alpha''(t) + B_2}{B_2} = 1 - \frac{[S'']/[S']}{\exp(-n_{ION} \cdot \Delta\tilde{\mu}_{ION} \cdot /RT)} \quad (11)$$

varying from 1 at $[S''] = 0$ to 0 at equilibrium. If the system is far from equilibrium as in Fig. 2, Eq. (11) assumes a value near unity, and Eq. (10) can be simplified by the approximation $\ln(1 + x) \approx x$, for small x, yielding

$$\frac{[S''(t)]}{[S']} \approx \frac{C_T \cdot k_{cat}(\text{influx}) \cdot \Delta t}{[S'] + K_T(\text{influx})} \quad (12)$$

where the combinations of A_1, A_2, B_1 and B_2 have been replaced by the appropriate experimental parameters of k_{cat} and K_T (*cf.* Wright, 1986*a*). Thus, solute accumulation by a strictly coupled cotransporter can be described by an equation resembling Eq. (3). The only requirement is that the rate of net transport be slow far from equilibrium. Equation (12) also suggests that the hyperbolic dependence of $[S'']$ on $[S']$ should be observed at any given time, not just in the apparent steady state. Observations by Kepes (1971) demonstrate this is so, thus suggesting that product inhibition is the correct explanation for the disequilibrium between $\Delta\tilde{\mu}_{H^+}$ and the accumulation of galactoside at finite times in this system.

Advancing product inhibition as an explanation for the behavior described by Eq. (3) implies that there is no nonequilibrium steady state. The internal concentration of solute slowly increases until, in the limit of infinite time, Eq. (2) is satisfied. Inspec-

tion of Fig. 2 discloses that this increase might be difficult to detect experimentally. A tangent to the curve for $\alpha' = 1$ at 5×10^4 sec has a slope less than 0.2% of the uptake curve measured under initial-velocity conditions ($t \approx 10$ sec). Thus, the slow rise in $[S'']$ could go unnoticed with an experimental error of even a few per cent and may be mistaken for a steady state.

Reasons for Slow Rates of Net Transport of Solute

In the case of galactoside : H^+ cotransport, two factors are responsible for slow rates of net transport (Wright et al., 1985; Wright, 1986*b*): The cotransporter retains high affinity for galactosides and H^+ on the cytoplasmic side of the membrane in the presence of $\Delta\tilde{\mu}_{H^+}$, and $\Delta\psi$ hardly changes k_c^+ and k_c^-. Thus, as the internal concentration of galactoside rises, the ternary complex CHG'' is easily formed. The rate of net influx is given by

$$\frac{d[S'']}{dt} = k_c^+ [\text{CHS}'] - k_c^- [\text{CHS}''] . \tag{13}$$

Net influx will approach zero while the system is far from equilibrium, because the concentration of CHS'' is high and k_c^+ and k_c^- are similar. This is an extreme form of product inhibition. However, the cotransporter rapidly exchanges H^+ and galactoside across the membrane under these conditions (Wright, 1986*b*). Cotransporters must not necessarily evince such strong product inhibition. If the effect of imposing $\Delta\psi$ were to increase k_c^+ and decrease k_c^- or to increase K_S'' according to

$$\frac{k_c^+}{k_c^-} = \left(\frac{k_c^+}{k_c^-}\right)_{\Delta\psi=0} \exp(-F\Delta\psi/RT) \tag{14}$$

or

$$K_S'' = (K_S'')_{\Delta\psi=0} \exp(-F\Delta\psi/RT) \tag{15}$$

as required by thermodynamic considerations, the rate of net influx at finite times could be higher than the observed rate of galactoside : H^+ cotransport (Fig. 2). Thus, the behavior described in Eq. (3) could, in principle, be avoided or diminished by mutation, if this were advantageous to a cell.

The Possible Existence of Catalytic Inefficiency in Other Cotransporters

Limiting the maximal level of a cotransported solute by catalytic inefficiency may be advantageous to a cell or organelle. Because this type of control is exerted without resorting to leaks, no energy is wasted. Inspection of the literature reveals at least two other cases where this behavior may be anticipated.

Komor and Tanner (1974), studying hexose : H^+ cotransport in the green alga *Chlorella vulgaris*, noted that the accumulation of 6-deoxyglucose decreased with decreasing $[H^+]$. One possible explanation is that the system possesses an inner leak via k_S. Raising the pH would titrate the sugar transporting complex from CHS' (k_c^+, with $n_{H^+} = 1$) to CS' (k_S^+, with $n_{H^+} = 0$). Alternatively, the rate of net influx may decrease with increasing pH. The values of k_{cat} and K_T for active transport are expected to be dependent on the concentration of the cotransported ion. In fact, the decrease in the maximal velocity of 6-deoxyglucose : H^+ cotransport exactly parallels the decrease in the accumulation (*see* Table 1 of Komor & Tanner, 1974). Thus at higher pH, net transport may be slow.

D-glucose : Na^+ cotransport in the intestinal brush-border membrane can be inhibited by internal D-glucose or Na^+ (Kessler & Semenza, 1983; Dorando & Crane, 1984; Kaunitz & Wright, 1984). Thus, net influx of D-glucose will be inhibited as $[Na^{+\prime\prime}]$ increases. This may prevent depolarization of the membrane by keeping the accumulation of D-glucose far from its potential equilibrium value.

Finally, it is interesting to note that de Meis and Inesi (1985) have suggested the Ca^{2+}-pumping ATPase of sarcoplasmic reticulum is also kinetically regulated to remain far from equilibrium under certain conditions. The accumulation of Ca^{2+} is thermodynamically determined by the free energy of hydrolysis of ATP. Therefore, in the presence of leaky vesicles where $[Ca^{2+\prime}] = [Ca^{2+\prime\prime}]$, ATP should be continuously hydrolyzed. However, at high concentrations of Ca^{2+} and phosphate (a product), the net hydrolysis of ATP is very slow. Thus, the Ca^{2+}-pumping ATPase is kinetically designed not to transport Ca^{2+} rapidly at the expense of ATP under certain conditions.

All protein-catalyzed reactions must exhibit product inhibition, because at equilibrium the net flux or conversion of substrates must be zero. However, some systems may be outfitted with kinetic traps (e.g., high affinity for product or fast rates for the reverse reaction), which cause the reaction catalyzed to remain far from equilibrium.

Discussion

Ion : solute cotransporters utilize the energy available from the transmembrane difference of the electrochemical potential of the ion to drive the accu-

mulation of the solute. When solute is added to a suspension of cells or organelles there is an initial period of uptake of solute, linear in time, followed by a progressive decrease in the rate of net transport due to product inhibition by internal ion and solute. If the fluxes of ion and solute are tightly coupled, the rate of net transport becomes zero ($d[S'']/dt = d[\mathrm{ION}'']/dt = 0$) only when the equilibrium of Eq. (2) is attained.

Here an alternative to leaks for explaining low accumulations of solute is suggested. Cotransporters may be kinetically so designed that the rates of net solute and ion fluxes, strictly coupled via the cotransporter, approach zero under certain circumstances, while the system is quite far from equilibrium. In principle, such cotransporters can attain the equilibrium of Eq. (2), but only in the limit of infinite, and biologically irrelevant, time. Particular constellations of cotransport parameters (Fig. 1) will evoke product inhibition more strongly than others, so that this behavior is biologically selectable.

In analogy to the thermodynamic inefficiency (Eq. (9)) associated with leaks, extremely small rates of net transport far from equilibrium could be called catalytic inefficiency E_c. A quantitative measure of the catalytic efficiency could be the half time $t_{1/2}$ for the attainment of equilibrium according to

$$E_c = 1/t_{1/2} \tag{16}$$

where $t_{1/2}$ can be calculated from Eq. (10). This definition is useful because the variability of E_c, even for a single cotransporter, is emphasized: E_c is a function of C_T, $[\mathrm{H}^{+\prime\prime}]$, and $[S']$, for example.

Catalytic inefficiency is proposed as the reason for low accumulations of hydrophilic disaccharides such as lactose and GalSGal via the galactoside : H^+ cotransporter of *E. coli*. At low external concentrations of galactoside, large accumulations are attained. At high external concentrations of galactoside, low accumulations are attained. The net result is that the internal concentration of galactoside varies over a narrower range than if the equilibrium of Eq. (2) were achieved. Thus, a variable catalytic efficiency could be a crude form of homeostatic control.

Low catalytic efficiency in cotransport (Eq. (12)) has consequences for the interpretation (*i*) of accumulations in the apparent steady state in terms of a stoichiometry, (*ii*) of selection procedures or growth phenotypes for mutants, or (*iii*) of the effects of chemical modifications. If an ion : solute cotransporter is catalytically inefficient, the accumulation ratio observed at finite times will depend on $[S']$, C_T, and k_{cat}. This has the following consequences:

Any effect that alters the number of functioning proteins such as variable induction, mutations in repressors or enhancers, mutations reducing incorporation, or chemical modification (*see* discussion in Neuhaus & Wright, 1983) will lead to lower accumulation at finite times. Phenotypes based on the ability to exclude a poisonous substrate (such as *p*-nitrophenyl-β-D-thiogalactoside) or to grow on a substrate (such as lactose, melibiose, or maltose) are generally determined at millimolar levels of solute where Eq. (3) or (12) and not Eq. (2) applies. Also, in reconstituted systems, the density of cotransporters is usually lower than that in the cell. Therefore, solute accumulation in the apparent steady state at low levels of cotransporters could yield a low estimate of the stoichiometry (*cf.* Wright & Overath, 1984). On the other hand, cloning a mutated gene onto a multicopy plasmid would lead to overproduction and to increased rates of influx. Therefore, suggestion that the *lac*Y^{UN} mutants are not uncoupled but catalytically inefficient (Wright & Seckler, 1985) could be tested by cloning this gene.

Any effect that decreases the turnover number of active transport such as mutations (Wright & Seckler, 1985), chemical modification (*cf.* Neuhaus & Wright, 1983), interaction with regulatory factors (*cf.* Nelson, Wright & Postma, 1983) or antibodies (Carrasco et al., 1984), the presence of synthetic or simply foreign phospholipids (Seto-Young, Chen & Wilson, 1985), or lower temperature, (Therisod, Weil & Shechter, 1978; *cf.* Overath et al., 1979) will lead to lower accumulation at finite times. Therefore, mutants which fail to grow upon or utilize certain solutes rapidly or exhibit low accumulation of solute (*cf.* Brooker, Fiebig & Wilson, 1985; Neuhaus et al. 1985; Padan et al., 1985) should not automatically be thought to possess inner leaks (i.e., be uncoupled), for example. Also, because turnover numbers in vesicles and proteoliposomes are frequently much smaller than those in cells (Wright et al., 1986; Wright & Overath, 1984), low accumulations may be more pronounced in these systems.

Finally, for the sake of completeness the role of the passive diffusion (outer leak, P_S) in determining the accumulation of solute in some instances must be acknowledged.

Measurements of P_S and the parameters of active transport are required to determine the extent to which the passive diffusion of solute influences the accumulation in each case.

Additionally, this investigation agrees with a conclusion of Sanders et al. (1984, Fig. 18) based on

a theoretical analysis of kinetic models for cotransport. In catalytically inefficient systems, force-flow relationships of the sort

$$\frac{d[S'']}{dt} = \text{constant} \cdot (n_{ION} \cdot \Delta\bar{\mu}_{ION} + \Delta\mu_S) \qquad (17)$$

cannot readily be established. The simulation in Fig. 2 demonstrates that under these conditions the flow approaches zero when the residual driving force is still very large. To the extent that ion-pumping ATPases or oxidoreductases exhibit similar kinetic designs, force-flow relationships like Eq. (17) will not be observed. Such systems cannot accurately be described using thermodynamics of irreversible processes.

In summary, low accumulations of solute should not always be interpreted in terms of incomplete coupling of ion and soluble fluxes via a cotransporter. Certain combinations of rate and dissociation constants produce conditions where net solute influx approaches zero far from equilibrium even in tightly coupled systems. Therefore, mutant and chemically modified cotransporters must be subject to extensive kinetic analysis to identify the cause of low accumulations of solute. Catalytic inefficiency because of strong product inhibition can mimic leaks.

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