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EFFECT OF HIGH SUBSTRATE CONCENTRATIONS ON ACTIVE TRANSPORT PARAMETERS

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Summary

A general model is described to account for the observation that steady-state accumulation ratios (mainly of non-electrolytes) decrease with increasing solute concentration, frequently reaching values of less than unity. Three variants of the model are treated, all of them including the assumption that the immediate supply of the source of energy is limited and its local concentration is appreciably reduced by its interaction with the transport system. Consequences of this assumption for the kinetic parameters of the initial rate of transport are analyzed and compared with experimental data.

Introduction

Active transport of non-electrolytes found in the vast majority of cells is driven by energy derived either from a high energy compound or directly from an oxidative reaction, or from an electrochemical gradient of H^+ or Na^+, this last-named mechanism being identified in an ever-increasing number of cases as refined techniques are applied to the task [1,2]. No matter what the source of energy, a steady state is reached in the active transport of non-metabolizable solutes, characterized by an accumulation ratio, $[S_{II}]/[S_I]$, relating the intracellular to the actual extracellular concentration. It has been repeatedly observed that this accumulation ratio is not constant over the whole concentration range used, decreasing at high concentrations either toward or even below unity [3—7] (Fig. 1). This decrease was originally attributed to the presence of a parallel diffusional leak, such that the substrate was transported into the cell by a saturable system and leaked out by simple diffusion [8] or, more realistically, that there was an ambidirectional leak present [9]. However, saturable efflux has been observed in many cells [10] and, moreover, in the case of molecules of molecular weight greater than 150—200 there does not appear to be a diffu-

sional pathway in most microbial cells [3,11]. Saturable carrier models of active transport, based either on equilibrium [12] or on steady-state [13,14] assumptions did not attempt to account for the decrease in the accumulation ratio. Only Silverman and Goresky [15], in an elegant treatment, predicted a trend of the $[S_{II}]/[S_I]$ toward unity at high substrate concentrations. However, firstly they worked with an equilibrium model and, secondly, they assumed finite mobility of the non-energized carrier-substrate complexes so that they could not attain values of $[S_{II}]/[S_I]$ below unity. The attempt described by Kotyk and Janáček [16], based on an excess-substrate inhibition, appears somewhat unlikely in the light of what is known about the molecular basis of transport systems.

It would thus appear that a more universal approach encompassing the various possibilities is required, as suggested by Rosenberg and Wilbrandt [12]. An attempt in this direction is the subject of the present communication.

Theory

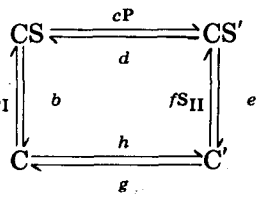
We shall discuss here three types of energy-driven transport, their common characteristic being an obligatory coupling with the energy source, such that in the absence of the source or in the case of its unavailability there would be no transport whatever.

Another important assumption required for the derivation is that the available concentration of the energy-donating compound (or of the driving ion) is limited and locally lower than the concentration of the carrier (it is assumed here for simplicity that the substrate binds to a protein molecule in the membrane and undergoes translocation in the complex, although more complicated models may be more realistic). The relatively low level of the energy source is easily envisaged as being due to steric hindrance, tortuous diffusion path to the carrier site, etc. This limitation has the consequence, analogies for which exist in the kinetics of soluble enzymes, that the actual concentration of the energy source decreases with increasing concentration of substrate or of the carrier · substrate complex, just as the amount of enzyme available for a chemical reaction is in fact $[E_t] - [ES]$ and that of substrate $[S_t] - [ES]$, with appropriately more complicated terms for two-substrate reactions, such as is that considered here in a transport context.

The three models to be treated here are basically that of Jacquez [13] and those described for secondary active transport by Schultz and Curran [17].

In the first model

I



II

it is assumed in the present treatment that the energy source denoted as P changes the conformation of the carrier · substrate complex into CS′ and, at the same time, exposes it to the other side of the membrane. (No affinity change of

the carrier for its substrate need be involved in the process.) It then dissociates into free carrier and substrate, this free carrier being then reconverted to C while being translocated to the outer face of the membrane. (This model was briefly treated in [18].)

To calculate the flow of S from side I to side II we use one of the modifications of the King-Altman method [19], for example that due to Fromm [20]. The concentrations of the various carrier forms are then expressed as fractions of the total carrier concentration $[C_t]$, using their determinants, as follows:

$$[C]/[C_t] = \frac{bg(d+e) + ceg[P] + bdf[S_{II}]}{\Sigma} \tag{1a}$$

$$[CS]/[C_t] = \frac{ag(d+e)[S_I] + df[S_{II}]([S_I] + h)}{\Sigma} \tag{1b}$$

$$[CS']/[C_t] = \frac{ac[S_I][P](f[S_{II}] + g) + fh[S_{II}](c[P] + b)}{\Sigma} \tag{1c}$$

$$[C']/[C_t] = \frac{bh(d+e) + ce[P](a[S_I] + h)}{\Sigma} \tag{1d}$$

where Σ is equal to the sum of all four numerators of the above expressions.

Since the flow is given by

$$J_s = c[P][CS] - d[CS'] \tag{2}$$

it will cease ($J_s = 0$) when $c[P][CS] = d[CS']$ and hence (from Eqns. 1b and 1c)

$$[S_{II}]/[S_I] = \frac{aceg}{bdfh}[P] \tag{3}$$

so that the accumulation ratio is a function of [P].

Now the total actual concentration of the energy source, $[P_t]$, is given by $[P_t] = [P] + [CS']$ and we may proceed to evaluate [P] from this equation. It appears as the positive root of a quadratic equation, thus:

$$[P] = \frac{\delta[P_t] - \alpha[C_t] - \gamma + \sqrt{(\delta[P_t] - \alpha[C_t] - \gamma)^2 + 4\delta(\gamma[P_t] - \beta[C_t])}}{2\delta} \tag{4}$$

with $\alpha = acg[S_I] + acf[S_I][S_{II}] + cfh[S_{II}]$; $\beta = bfh[S_{II}]$;
$\gamma = bdg + beg + beh + (adg + aeg)[S_I] + (bdf + bfh + dfh)[S_{II}] + adf[S_I][S_{II}]$;
$\delta = ceg + ceh + (ace + acg)[S_I] + cfh[S_{II}] + acf[S_I][S_{II}]$.

On introducing this root into Eqn. 3 we obtain a cubic equation in both $[S_I]$ and $[S_{II}]$ of the form

$$(A + B[S_I])[S_{II}]^3 + (C + D[S_I] + E[S_I]^2)[S_{II}]^2 +$$
$$+ (F[S_I] + G[S_I]^2 - H[S_I]^3)[S_{II}] - J[S_I]^2 - K[S_I]^3 = 0 \tag{5}$$

where A—K are composite coefficients containing either some or all of the partial step constants (a—h) and, in the case of D—H, also $[P_t]$; in the case of D,E, and G also $[C_t]$.

Eqn. 5 can be solved numerically for different combinations of the constants. As an example, Fig. 2 has been constructed using plausible values for the

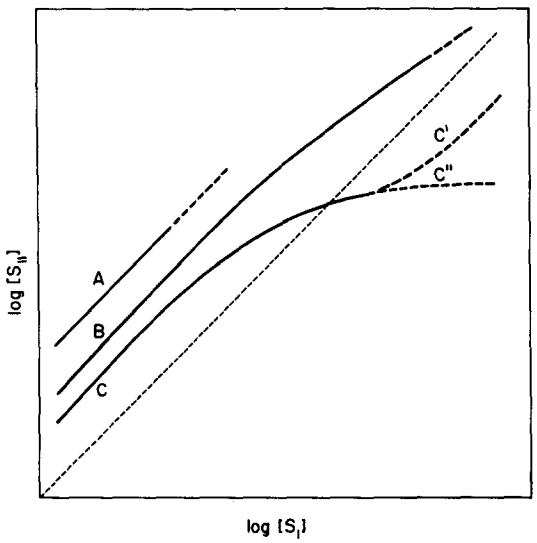


Fig. 1. Different types of dependence of $[S_{II}]$ on $[S_I]$ in a steady state observed experimentally. A, Theoretical constant-ratio dependence; B, accumulation ratio tends toward unity; C, accumulation ratio decreases below unity and then remains constant (C') or keeps decreasing indefinitely (C").

constants (their physical dimensions are not given but it is understood that b, c, d, e, g, and h are in s^{-1}, a and f are in $M^{-1} \cdot s^{-1}$). It will be seen that even for identical intrinsic dissociation constants at the two membrane faces ($b/a = e/f = 10$) there is a gradual shift from $[S_{II}]/[S_I] > 1$ to $[S_{II}]/[S_I] < 1$ as $[S_I]$ increases. In fact, under a variety of conditions (e.g., for very small $[P_t]$) this will be true even if, as is often assumed, the intrinsic dissociation constant of the carrier · substrate complex at the inner face is greater than at the outer face ($e/f > b/a$).

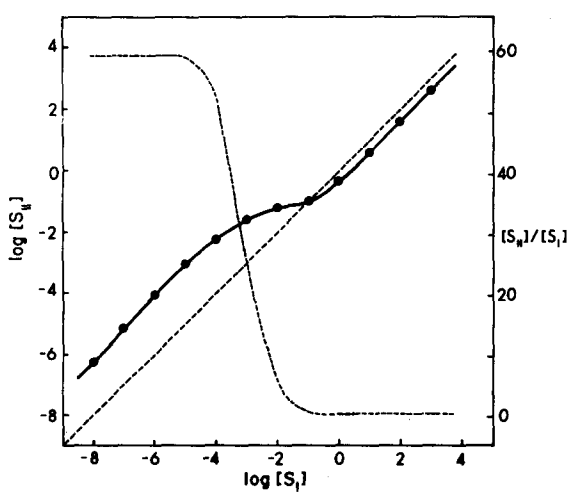
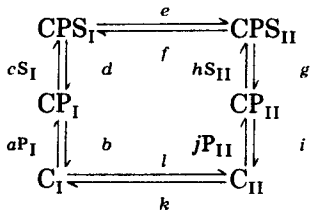


Fig. 2. Dependence of intracellular steady-state concentration (solid line) and of the accumulation ratio (broken line) on extracellular solute concentration, according to Model I, for $a = 100$, $b = 10$, $c = 10$, $d = 0.1$, $e = 10$, $f = 100$, $g = 1$, $h = 1$, $[C_t] = 2$, $[P_t] = 0.5$.

The second model



attempts to describe a symport mechanism where P is the driving ion. To facilitate the derivations let us assume that the CP complex is immobile and that $[P_{II}]$ is constant (i.e. its changes are quickly balanced by an intracellular buffering system). Hence we may include it in the j constant in further calculations. Also the amount of CPS_{II} and CP_{II} need not concern us because the two forms are separated from the extracellular energy source by the membrane barrier and, indeed, are in equilibrium with intracellular P. In this case $[P_t] = [P] + [CP_I] + [CPS_I]$.

Following the same procedure as above we arrive at an analogy of Eqn. 8, with different meanings of the *A-H* coefficients (e.g., both $[C_t]$ and $[P_t]$ occur here in *D—H*). Fig. 3 shows the results of two sample calculations. In the first, the asymmetry of the system resides in different intrinsic dissociation constants of the CP complex at the two membrane sides ($i/j > b/a$). In contrast with the first model the $[S_{II}]/[S_I]$ ratio keeps decreasing and $[S_{II}]$ is constant at high concentrations of S_I.

The second set of values was chosen so as to bring the asymmetry into the translocation reaction, viz. to make $e >> f$. This is the situation that is likely to obtain when the membrane potential becomes the driving force, such that the

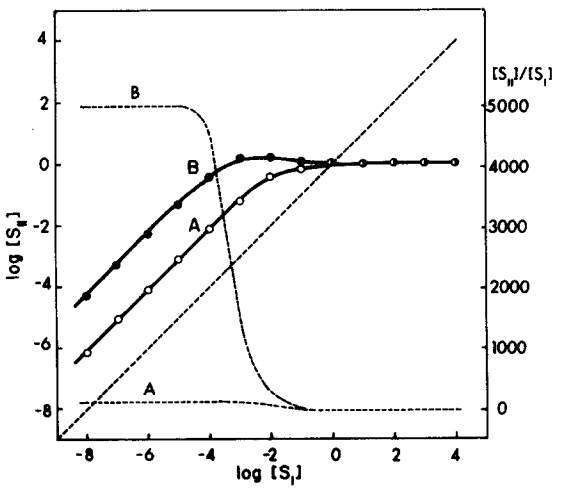
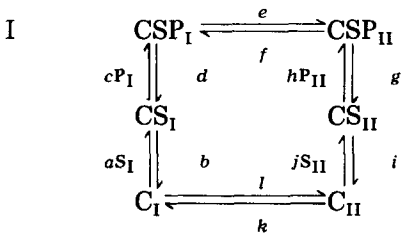


Fig. 3. Dependence of intracellular steady-state concentration (solid line) and of the accumulation ratio (broken line) on extracellular solute concentration, according to Model II, for (curve A/curve B) a = 1000/1000, b = 1/1, c = 100/100, d = 1/1, e = 1/10, f = 1/0.1, g = 1/1, h = 100/100, i = 1/1, j = 0.01/0.01, k = 1/1, l = 1/1, $[C_t]$ = 2/2, $[P_t]$ = 0.5/0.01.

positively charged CSP complex is attracted toward the negative inner face of the membrane [21]. An important restriction is required if a constant decline of the accumulation ratio is to be obtained, namely, that $f[C_t] > e[P_t]$. Now, since $e > f$, the ratio $[P_t]/[C_t]$ must be rather small.

The third model



again may be considered as a symport mechanism but here the "activation" follows the binding of the transported solute. The coupling is obligatory for movement so that the CS form is immobile. As in Model II, the intracellular concentration $[P_{II}]$ is considered constant and will be included in rate constant h. Here then $[P_t] = [P] + [CPS_I]$ and Eqn. 5 has both $[C_t]$ and $[P_t]$ in coefficients D, E and G.

Fig. 4 shows an example of $[S_{II}]$ dependence on $[S_I]$ for a plausible combination of constants. If the energy-dependent asymmetry resides in the lower dissociation constant of the CSP complex outside rather than inside, the behaviour of $[S_{II}]$ at high solute concentrations resembles that of Model I (apparently the similarity arises from the fact that P combines with the system only after S). If the binding of P causes an asymmetry in the translocation constants ($e > f$) there are even more rigorous limitations than in Model II to obtain a constant decline of $[S_{II}]/[S_I]$.

In all cases discussed above the available "concentration" of P in the steady

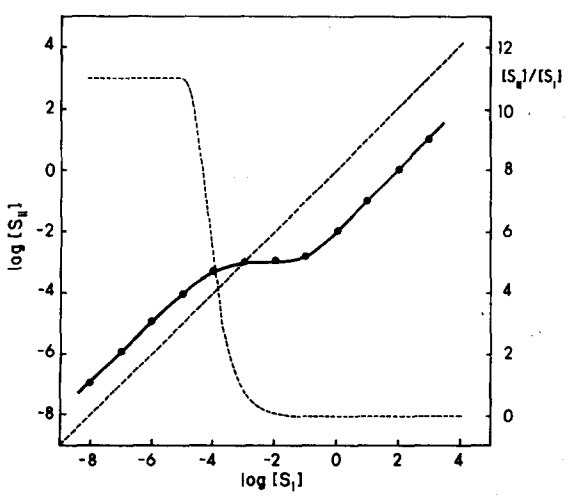


Fig. 4. Dependence of intracellular steady-state concentration (solid line) and of the accumulation ratio (broken line) on extracellular solute concentration, according to Model III, for $a = 100$, $b = 10$, $c = 1000$, $d = 1$, $e = 1$, $f = 1$, $g = 1$, $h = 1$, $i = 10$, $j = 100$, $k = 1$, $l = 1$, $[C_t] = 2$, $[P_t] = 0.02$.

state decreases with increasing concentration of solute S. However, the fact that [P] is a function of solute concentration is manifested even in the expressions for apparent K_T and J_{max} of the initial rate of transport. Thus for Model I we have

$$J_{max} = [C_t] \frac{ceg[P]}{g(d+e) + c[P](e+g)}$$

and

$$K_T = \frac{(g+h)[b(d+e) + ce[P])]}{a[g(d+e) + c[P](e+g)]}$$

For Model II

$$J_{max} = [C_t] \frac{aegik[P]}{a[P][eg(i+j+k) + ik(e+f+g)] + egi(k+l)}$$

and

$$K_T = \frac{(df+dg+eg)(bik + bil + bjl + aik[P])}{ac[P][eg(i+j+k) + ik(e+f+g)] + cegi(k+l)}$$

For Model III

$$J_{max} = [C_t] \frac{cegi[P]}{c[P](eg+eh+ei+fh+fi+gi) + df(h+i) + gi(d+e)}$$

and

$$K_T = \frac{b(k+l)[df(h+i) + gi(d+e)] + cegi[P](a+k+l)}{ak[c[P](eg+eh+ei+fh+fi+gi) + df(h+i) + gi(d+e)]}$$

The initial amount of [P], i.e. after the carrier cycle has reached the steady state (not to be confused with the steady state of the transport system at the end of incubation), can be computed from expressions 1a—1d, assuming $[S_{II}] = 0$, and from Eqn. 4 (or analogies for Models II and III).

Thus, using the values of Figs. 2—4, with increasing concentration of S_I the J_{max} is seen to decrease in Model I, increase in Model II and not change in Model III; the K_T is seen to increase in Model I, decrease in Model II, and not change in Model III. It would be wrong to assume, however, that a graphical representation, either of the reciprocal type (Lineweaver and Burk) or using the direct linear plot [22] the apparent values would correspond to the above pattern. Since the commonly used plots are based on the assumption of linearity and uniqueness of the kinetic parameters an attempt to read them from a representation of an intrinsically nonlinear relationship is erroneous. Still, as a qualitative indicator of the mechanism possibly involved it may be helpful to know that with increasing concentrations of S_I the graphically derived value of J_{max} increases in Model I and decreases in Model II while the apparent K_T increases in Model I and decreases in Model II. The differences may not be dramatic but with the values of rate constants used here a difference by a factor of three is easily arrived at.

Discussion

The present approach to answering in kinetic terms the question of why the accumulation ratio decreases with concentration yields qualitatively acceptable results. The possibility of distinguishing between Models I and III, and Model II, based on the behaviour of $[S_{II}]$ at high values, may not be as useful as it appears here. Since it concerns concentrations that in most cases are near the solubility limit of the transported substance it is difficult to go far enough to the right on the $[S_I]$ scale to be certain about the distinction. Still, it is readily possible to distingusih between a system with obligatory coupling and one without it for in the former the accumulation ratio can decrease below unity while in the latter it cannot (even the non-energized CS complex can move across the membrane and thus bring about diffusion equilibrium of the transported solute).

The consequence of the models with regard to the kinetic parameters of transport is borne out by a number of observations of nonelectrolyte transport in yeast species [23] that both the J_{max} and the K_T increase as read from a graph, suggesting the operation of Model I. It should be noted that the presence of a leak accompanying the saturable transport could give somewhat similar results but in such a case, in a Lineweaver-Burk plot, the curve would tend toward the origin while in the energy-limited case such behaviour is not expected (nor is it observed in the experimental case reported).

References

1 Mitchell, P. (1977) in Microbial Energetics (Haddock, B.A. and Hamilton, W.A., eds.), pp. 383—423, Cambridge University Press, Cambridge, U.K.
2 Harold, F.M. (1972) Bacteriol. Rev. 36, 172—230
3 Kotyk, A. and Höfer, M. (1965) Biochim. Biophys. Acta 102, 410—422
4 Novotny, C.P. and Englesberg, E. (1966) Biochim. Biophys. Acta 117, 217—230
5 Kepes, A. (1970) in Current Topics in Membranes and Transport (Bronner, F. and Kleinzeller, A., eds.), Vol. 1, pp. 101—134, Academic Press, New York
6 Deák, T. and Kotyk, A. (1968) Folia Microbiol. 13, 212—220
7 Christensen, H.N. and Riggs, T.R. (1952) J. Biol. Chem. 194, 57—68
8 Stein, W.D. (1967) The Movement of Molecules across Cell Membranes, p. 232, Academic Press, New York
9 Heinz, E. (1954) J. Biol. Chem. 211, 781—790
10 Manno, J.A. and Schachter, D. (1970) J. Biol. Chem. 245, 1217—1223
11 Canh, D.S., Horák, J., Kotyk, A. and Říhová, L. (1975) Folia Microbiol. 20, 320—325
12 Rosenberg, T. and Wilbrandt, W. (1963) J. Theor. Biol. 5, 288—305
13 Jacquez, J.A. (1964) Biochim. Biophys. Acta 79, 318—328
14 Schachter, H. (1972) in Metabolic Transport (Hokin, L.E., ed.), pp. 1—15, Academic Press, New York
15 Silverman, M. and Goresky, C.A. (1965) Biophys. J. 5, 487—509
16 Kotyk, A. and Janáček, K. (1975) Cell Membrane Transport, 2nd edn., pp. 151—152, Plenum Press, New York
17 Schultz, S.G. and Curran, P.F. (1970) Physiol. Rev. 50, 637—718
18 Kotyk, A. (1976) Wiss. Z. Humboldt Univ. Math.- Naturwiss. Reihe 25, 39—43
19 King, E.L. and Altman, C. (1956) J. Phys. Chem. 60, 1375—1378
20 Fromm, H.J. (1970) Biochem. Biophys. Res. Commun. 40, 692—697
21 Geck, P. and Heinz, E. (1976) Biochim. Biophys. Acta 443, 49—53
22 Eisenthal, R. and Cornish-Bowden, A. (1974) Biochem. J. 139, 715—720
23 Kotyk, A. and Michaljanicŏvá, D. (1977) Folia Microbiol. 22(6), in the press