REFERENCES

1. Simoni, R. D., Nakazawa, T., Hays, J. B., and Roseman, S. (1973) J. Biol. Chem. 248, 592-940
2. Hays, J. B., Simoni, R. D., and Roseman, S. (1973) J. Biol. Chem. 248, 941-956
3. Simoni, R. D., Hays, J. B., Nakazawa, T., and Roseman, S. (1973) J. Biol. Chem. 248, 957-966
4. Roseman, S. (1969) J. Gen. Physiol. 54, 1388
5. Tanaka, S., and Lin, E. C. C. (1967) Proc. Nat. Acad. Sci. U. S. A. 52, 913
6. Tanaka, S., Fraenkel, D., and Lin, E. C. C. (1967) Biochem. Biophys. Res. Commun. 27, 63
7. Fox, C. F., and Wilson, G. (1968) Proc. Nat. Acad. Sci. U. S. A. 69, 988
8. Simoni, R. D., Levinthal, M., Kundig, F., Kundig, W., Anderson, B., Hartman, P., and Roseman, S. (1967) Proc. Nat. Acad. Sci. U. S. A. 65, 1965
9. Sailer, M. H., Jr., Simoni, R. D., and Roseman, S. (1970) J. Biol. Chem. 245, 5570
10. Roseman, S. in Metabolic Pathways (Hokin, L. E., ed) Vol. VI, Academic Press, New York, in press
11. Egan, J. B., and Morse, M. L. (1965) Biochim. Biophys. Acta 109, 172
12. Kennedy, E. P., and Scarborough, G. A. (1967) Proc. Nat. Acad. Sci. U. S. A. 65, 225
13. Hengstenberg, W., Penberthy, W. K., and Morse, M. L. (1968) Fed. Proc. 27, 643
14. Simoni, R. D., Smith, M., and Roseman, S. (1968) Biochim. Biophys. Res. Commun. 31, 804

APPENDIX

ON THE INTERPRETATION OF MICHAELIS CONSTANTS FOR TRANSPORT

Harry Schachter

From the Department of Biochemistry, University of Toronto, Toronto, Canada

Correlations between the dissociation constant of a solute for a specific solute-binding protein and the apparent Michaelis constant for the transport of the solute into intact cells have been invoked to provide evidence for the hypothesis that the solute-binding protein is a rate-limiting component of the transport process (1). However, the accompanying papers (2-4) show little or no correlation between constants derived from the following three types of measurement: (a) the dissociation constant of the lactose-Enzyme II lac complex (the Kn values rather than Ping Pong Bi Bi (5, 6). The mechanism for such a linear reaction can be represented as follows:

\[
\Pi^{\text{mon}} \xrightleftharpoons[k_d]{k_s} S \Pi^{\text{mon}}
\]

(1)

where \( S \) is concentration of sugar (i.e. lactose) and \( k_s \) and \( k_d \) are the respective rate constants, then:

\[
K_D = k_d / k_s
\]

(2)

where \( E \) represents free enzyme concentration, \( E S_1 \), \( E S_2 S_2 \), and \( E P_2 \) represent the concentrations of the various substituted enzymes, \( S_1, S_2 \) are substrate concentrations, \( P_1, P_2 \) are product concentrations, and \( k_1, k_2, k_3, k_4, k_5, k_6, k_7, k_8, k_9, k_10, k_11 \) are the respective rate constants.

Let \( E_i \) represent the total enzyme concentration:
\[ E_t = E + ES_1 + ES_2 + EP_3 \]  
(4)

If one makes the steady state assumption (5, 6), the following general rate equation for Reaction 3 can be derived.

\[ v = \frac{(k_{-3}k_3k_6k_8S_2 - k_3k_6k_8k_9P_1P_2)E_t}{\Delta} \]  
(6)

where \( v \) is the rate of reaction through the system at time \( t \) (see Equation 6 for value of \( \Delta \)).

\[ \Delta = k_3k_5(k_3 + k_6)S_1S_2 + k_3k_4(k_4 + k_5)S_1 + k_6k_8S_2 + k_3k_4(k_4 + k_5)S_1 + k_3k_4(k_4 + k_5)P_1P_2 + \\
k_6k_8S_2P_1 + k_6k_8S_2P_1 + k_6k_8S_2P_1 + k_6k_8S_2P_1 + k_6k_8S_2P_1 \]  
(7)

If it is assumed that \( P_1 = P_2 = 0 \) at time \( t = 0 \), then from Equations 5 and 6:

\[ v = \frac{k_3k_5k_6k_8S_1E_t}{\Delta} \]  
(8)

The rate equation for phosphorylation can be rewritten as follows.

\[ v = \frac{V_{\text{max}}S_1S_2}{S_1S_2 + K_3S_1 + K_4S_2 + K_3K_4} \]  
(9)

where

\[ K_1 = k_3k_5/[k_3(k_3 + k_6)] \]  
(10)

\[ K_2 = k_3(k_4 + k_5)/[k_3(k_3 + k_6)] \]  
(11)

\[ K_3 = k_6/k_1 \]  
(12)

\[ V_{\text{max}} = k_3k_5k_6S_1/k_3(k_3 + k_6) \]  
(13)

The apparent Michaelis constants are given by the following expressions.

\[ K_{(S_1)P-III'ac} = K_3 = k_3/k_6 \]  
(14)

\[ K_{(S_2)P-III'ac} = K_3 = k_3k_5/[k_3(k_3 + k_6)] \]  
(15)

\[ K_{(S_1S_2)P-III'ac} = K_3 - K_2/k_3(k_3 + k_6) \]  
(16)

\[ K_{(S_2S_2)P-III'ac} = K_3 - k_4(k_4 + k_5)/[k_3(k_3 + k_6)] \]  
(17)

where \( K_{S_1} \) and \( K_{S_2} \) represent the apparent Michaelis constants for \( S_1 \) and \( S_2 \), respectively.

Now, it has been shown in the accompanying papers (2-4) that the apparent Michaelis constant for phosphorylation of lactose, as the concentration of phospho-III'ac approaches zero, is \( 4 \times 10^{-7} \) M, and that the dissociation constant for the lactose-

1 When the reaction is initiated by addition of substrates \( S_1 \) and \( S_2 \), \( t \leq 0 \), \( v = v_1 \) (initial velocity), and \( P_1 \) and \( P_2 \) are both essentially zero. However, the phosphorylation assays in the accompanying papers were conducted by adding Factor III'ac and a phosphotransferase system to generate the substrate, phospho-III'ac. Thus at \( t = 0 \), one of the products (sugar-phosphate) was essentially absent but the other product (Factor III'ac) may not have been at zero concentration, and either \( P_1 = 0 \), \( P_2 \neq 0 \), or \( P_1 \neq 0 \), \( P_2 = 0 \). If one plots 1/\( v_1 \) versus 1/\( S_1 \) (or 1/\( S_2 \)) and then plots both the slopes and intercepts against 1/\( S_1 \) (or 1/\( S_2 \)), linear plots will result provided both \( P_1 \) and \( P_2 \) are zero. Such plots were made for the phosphorylation of lactose and its analogues TMG and IPTG by Enzyme II'ac; the plots were essentially linear indicating that it is probably a reasonable approximation to assume that most of the added Factor III'ac was phosphorylated at time \( t = 0 \).
of each process. The concentrations of the complexes between \( \Pi^{1\text{ac}} \) and sugar, sugar-P, and sugar plus phospho-\( \Pi^{1\text{ac}} \) are designated \( S_{1}, S_{2}, S, \) and \( S-P-\Pi^{1\text{ac}} \), respectively.

One can now use the steady state assumption and the procedure of King and Altman (7) to derive the general rate equation for movement of \( S \) from outside to inside the cell at any time \( t \):

\[
v = \frac{k_{1}k_{2}k_{3}k_{4}k_{5}k_{6}k_{7}k_{8}k_{9}}{\Delta'} \cdot \frac{(S-\Pi^{1\text{ac}})}{(S-P)(\Pi^{1\text{ac}})} \]

where \( \Delta' \) is a complex expression that can be determined as described by Wong and Hanes (6), and where \( \Pi^{1\text{ac}} \) is the total Enzyme \( \Pi^{1\text{ac}} \) concentration (free and substituted enzyme). If transport studies are conducted under conditions where progress curves are linear, i.e., within seconds of adding external sugar, then \( S-P \) is essentially zero with sugars such as TMG and IPTG, and \( v = v_{i} \) (initial velocity), where:

\[
v_{i} = \frac{k_{1}k_{2}k_{3}k_{4}k_{5}k_{6}k_{7}k_{8}k_{9}k_{10}}{\Delta'} \cdot \frac{(S-\Pi^{1\text{ac}})}{(S-P)(\Pi^{1\text{ac}})} \]

and where \( \Delta' \) is a somewhat simpler expression because \( (S-P) \) is zero. If one makes the further assumption that \( \Pi^{1\text{ac}} \) concentration is zero, the expression for \( \Delta' \) is given in Equation 22.

Therefore, the apparent Michaelis constants for transport will be given by

\[
(K_{p})_{\text{P-}\Pi^{1\text{ac}}} = \frac{k_{-1}k_{2}k_{3}k_{4}k_{5}k_{6}k_{7}k_{8}k_{9}}{k_{1}k_{2}k_{3}k_{4}k_{5}k_{6}} \]

(23)

and

\[
(K_{p})_{\text{P-}\Pi^{1\text{ac}}} = \frac{k_{1}k_{2}k_{3}k_{4}k_{5}k_{6}k_{7}k_{8}k_{9}}{k_{1}k_{2}k_{3}k_{4}k_{5}k_{6}} \]

(24)

Since the intracellular concentration of \( \Pi^{1\text{ac}} \) is probably not zero, the actual expressions for transport may be even more complex than given above.

It is obvious that the apparent Michaelis constants for transport given by Equations 23 and 24 are far more complex than the apparent Michaelis constants for phosphorylation given by Equations 18 and 19.

Since the above expressions were derived with certain assumptions that may not be wholly valid, the actual differences between the phosphorylation constants and the transport constants may be even more marked than indicated by Equations 18, 19, 23, and 24. Thus, for example, if the concentration of \( \Pi^{1\text{ac}} \) is not zero, or if phospho-\( \Pi^{1\text{ac}} \) actually exists in several forms (mono-, di-, and triphospho-\( \Pi^{1\text{ac}} \)), or if the intracellular concentration of phospho-\( \Pi^{1\text{ac}} \) is neither infinitely small nor large as assumed in Equations 23 and 24, or if the PTS in vitro does not function in the same manner as the PTS in vivo, both the phosphorylation and transport kinetics become more complex. It is thus evident that any agreement between the phosphorylation and transport constants must be fortuitous, or due to rather special conditions. For example, if \( k_{3} = k_{-2} = k_{4} = k_{-3} \) (a commonly used assumption), Equation 23 becomes identical with Equation 18 although Equation 24 remains more complex than Equation 19.

REFERENCES

1. **Hokin, L. E. (ed) (1972) Metabolic Pathways, Vol. VI, pp. 41-89, Academic Press, New York**
2. **Simoni, R. D., Nakazawa, T., Hays, J. B., and Roseman, S. (1973) J. Biol. Chem. 248, 932-940**
3. **Hays, J. B., Simoni, R. D., and Roseman, S. (1973) J. Biol. Chem. 248, 941-956**
4. **Simoni, R. D., Hays, J. B., Nakazawa, T., and Roseman, S. (1973) J. Biol. Chem. 248, 957-965**
5. **Cleland, W. W. (1963) Biochim. Biophys. Acta 67, 104, 173, 188**
6. **Wong, J. T-F., and Hanes, C. S. (1962) Can. J. Biochem. Physiol. 40, 763**
7. **King, E. L., and Altman, C. (1956) J. Phys. Chem. 60, 1375**
ON THE INTERPRETATION OF MICHAELIS CONSTANTS FOR TRANSPORT
Harry Schachter

J. Biol. Chem. 1973, 248:974-976.

Access the most updated version of this article at http://www.jbc.org/content/248/3/974.citation

Alerts:
  • When this article is cited
  • When a correction for this article is posted

Click here to choose from all of JBC’s e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/248/3/974.citation.full.html#ref-list-1