Protein Science (1992), *1*, 1661–1665. Cambridge University Press. Printed in the USA.

A kinetic model for binding protein-mediated arabinose transport

D.G. KEHRES

Department of Molecular Biology and Microbiology, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106

(RECEIVED May 27, 1992; REVISED MANUSCRIPT RECEIVED August 13, 1992)

Abstract

A kinetic model is presented based on the simplest plausible mechanism for bacterial binding protein-dependent transport. The transport phenotypes of the 18 variant arabinose-binding proteins analyzed by Kehres and Hogg (1992, *Protein Sci. 1*, 1652–1660) (wild type and 17 mutants) are interpreted to mean that in wild-type arabinose uptake the forward transport rate (k_{for}) greatly exceeds the dissociation rate (k_{und}) of a binding protein docked with the AraG:AraH membrane complex, and that k_{for} dominance is preserved in all of the binding protein surface mutants. The assumptions and predictions of the model are consistent with existing data from other periplasmic transport systems.

Keywords: kinetic model; periplasmic transport

Transport assays of the arabinose-binding protein (ABP) mutants reported by Kehres and Hogg (1992 [companion paper]) show two surprising properties. First, there is a striking correlation between the maximum initial uptake velocities V_{en} and the half-saturating arabinose concentrations K_{en} (Fig. 1). Such a pattern often occurs in simple one-step Briggs–Haldane enzyme reaction mechanisms when the rate of catalysis is rapid compared to the rate of substrate dissociation (Fersht, 1985). Second, none of the ABP mutations isolated totally abolish transport, although such mutants have been reported for other binding protein-dependent systems (see below). These preliminary findings suggest that the affinity of ABP for the AraG:AraH cytoplasmic membrane complex has little to do with the overall rate of transport, but the rates of the conformational changes that occur in the docked complex leading to translocation of arabinose are very important. The plausibility of this idea can be tested with the aid of an explicit kinetic model. A consensus exists among those studying binding protein-dependent transport (periplasmic transport) that both ligand acquisition by the binding protein and association of liganded binding protein with the membrane complex influence transport dynamics (Manson et al., 1985; Ames, 1986; Prossnitz et al., 1989), but no formal kinetic analysis of transport has yet appeared.

To address the questions raised by the ABP mutants, this report describes a two-step kinetic model that is consistent with existing knowledge of binding protein-dependent transport processes. A general relationship is

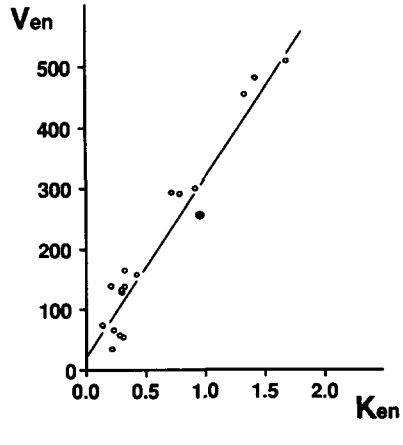

Fig. 1. Covariation of V_{en} and K_{en} for wild-type arabinose-binding protein (ABP) and 17 mutants with substitutions on the surface containing the mouth of the arabinose-binding cleft. The parameters in Table 1 of Kehres and Hogg (1992) have been plotted, and the line obtained by linear regression has been superimposed. The correlation coefficient $R^2 = 0.94$. The filled circle corresponds to wild-type ABP. V_{en} and K_{en} values are accurate to within about 12% and 20%, respectively.

Reprint requests to: D.G. Kehres, Department of Molecular Biology and Microbiology, School of Medicine, Case Western Reserve University, Cleveland, Ohio 44106.

found between the V_{en} and K_{en} measured in whole cell uptake assays and the four parameters that define the proposed individual steps of this process—the equilibrium dissociation constant K_d of ligand and binding protein, and the docking rate k_{doc}, undocking rate k_{und}, and forward translocation rate k_{for} of a liganded binding protein associating with the cytoplasmic membrane complex. The behavior of the ABP mutants is consistent with a limiting case of this model in which k_{for} greatly exceeds k_{und}. This limitation is proposed to be a genuine feature of the *araFGH* system, as all 17 cleft mouth surface mutants and wild-type ABP follow the pattern in Figure 1. A set of ribose-binding protein mutants derived in an independent study by Binnie et al. (1992 [companion paper]) has kinetic properties suggesting that k_{for} is not dominant in that system.

Results and discussion

The ABP mutant phenotypes have mechanistic implications

The strong correlation between the whole cell kinetic parameters V_{en} and K_{en} plotted in Figure 1 ($R^2 = 0.94$) suggests that both may be determined by the same mechanistic feature. When covariation of V_{max} and K_m is observed in a one-step enzymatic process obeying Michaelis–Menten kinetics, the two most likely mechanistic explanations are (1) that the irreversible forward rate k_2 is much greater than the dissociation rate k_{-1} of the enzyme-substrate complex ES—in such a case $K_m = k_2/k_1$ is proportional to $V_{max} = k_2[ES]$—or (2) that an alternate, nonproductive mode of binding between E and S (equilibrium dissociation constant K_s') competes with the catalytically productive binding mode (dissociation constant K_s)—in such a case V_{max} and K_m are each reduced by a factor of $(1 + K_s/K_s')$ but the ratio V_{max}/K_m remains unchanged (Fersht, 1985).

These mechanisms would ascribe the phenotypic effect of a series of mutations to two very different physical processes. It is not obvious that either mechanism leads to covariation in the whole cell V_{en} and K_{en} parameters measured for binding protein-dependent transport, since it is not a one-step process. To determine what mechanistic explanations can be applied to the behavior observed in Figure 1, an algebraic model of binding protein-dependent transport is presented. The model is derived from what are intended to be the simplest assumptions possible consistent with the current understanding of these systems.

A simple kinetic model

The two-step mechanism is defined in Figure 2. The binding protein P and its ligand A are assumed to come to equilibrium in the periplasm with a dissociation constant K_d (Assumption 1). The liganded binding protein PA is assumed to interact with the membrane complex M via a Briggs–Haldane process (i.e., a steady state in the concentration of $PA{:}M$) with a characteristic V_{max} and K_m (Assumption 2). V_{max} is assumed to be proportional to the forward rate k_{for}, uninfluenced by any subsequent steps such as the dissociation of A or P from M (Assumption 3), and K_m has its customary meaning as the dynamic affinity of PA for M (not its equilibrium dissociation constant K_s, which is discussed below). The translocation rate, v_0, of the membrane step is given by the Michaelis–Menten expression:

$$v_0 = V_{max} \frac{[PA]}{K_m + [PA]}. \quad (1)$$

Because transport into the cell is equivalent to the translocation rate v_0, uptake as a function of $[A]$, which is what whole cell uptake assays measure, can be obtained by writing $[PA]$ as a function of $[A]$ and substituting that expression into Equation 1. If the contribution of $[PA{:}M]$ to the total binding protein concentration $[P]_t$ is neglected (Assumption 4), then $[PA] = [P]_t - [P]$, and since $[PA] = K_d[P][A]$,

$$[PA] = [P]_t \frac{[A]}{K_d + [A]}. \quad (2)$$

$$K_s = \frac{k_{und}}{k_{doc}}$$

$$P + A \rightleftharpoons PA + M \underset{k_{und}}{\overset{k_{doc}}{\rightleftharpoons}} PA{:}M \xrightarrow{k_{for}} [\text{ Transport }]$$

Fig. 2. Simplest plausible mechanism for periplasmic transport.

$$K_d = \frac{[P][A]}{[PA]} \qquad K_m = \frac{[PA][M]}{[PA{:}M]} = \frac{k_{und} + k_{for}}{k_{doc}} \qquad V_{max} = k_{for}[M]_t$$

Combining Equations 1 and 2 yields

$$v_0 = V_{max} \frac{[P]_t[A]/(K_d + [A])}{K_m + [P]_t[A]/(K_d + [A])}$$

$$= V_{max} \frac{[P]_t[A]}{K_m K_d + K_m[A] + [P]_t[A]}. \quad (3a)$$

Inverting and separating variables yields

$$\frac{1}{v_0} = \frac{1}{V_{max}} \left(\frac{K_m K_d}{[A][P]_t} + \frac{K_m[A]}{[A][P]_t} + 1 \right)$$

$$= \frac{1}{[A]} \left(\frac{K_m K_d}{V_{max}[P]_t} \right) + \left(\frac{K_m + [P]_t}{V_{max}[P]_t} \right). \quad (3b)$$

Defining V_{en} and K_{en} as intercepts of Equation 3b yields

$$\frac{1}{V_{en}} = \frac{1}{v_0} \text{ when } \frac{1}{[A]} = 0; \quad (3c)$$

$$\frac{-1}{K_{en}} = \frac{1}{[A]} \text{ when } \frac{1}{v_0} = 0. \quad (3d)$$

Evaluating the intercepts yields

$$\frac{1}{V_{en}} = \frac{K_m + [P]_t}{V_{max}[P]_t}; \quad (3e)$$

$$\frac{-1}{K_{en}} = \frac{-(K_m + [P]_t)}{K_m K_d}. \quad (3f)$$

Solving these equations leads to two simple and useful relationships, Equations 4a and 4b, which define the model:

$$V_{en} = V_{max} \frac{[P]_t}{K_m + [P]_t}; \quad (4a)$$

$$K_{en} = K_d \frac{K_m}{K_m + [P]_t}. \quad (4b)$$

Equations 4 offer insight into how each stepwise process influences overall uptake and also serve to formalize the qualitative discussions of kinetics that have already been published. The validity of Assumptions 1–4 and the applicability of Equations 4 to periplasmic transport in general will be discussed after they have been applied to the arabinose case.

Application to arabinose transport

A feature of the control cultures that were assayed along with mutants provides the key to Figure 1. If individual V_{en}'s and K_{en}'s for the wild-type cultures averaged in that figure are plotted, fluctuations from one culture to another show the same covariation as was seen for the mutants (Fig. 3). It is clear from Equations 4 that covariation will indeed occur in the two-step periplasmic transport process, in the limiting condition that the forward rate k_{for} greatly exceeds the dissociation rate k_{und} of a $PA{:}M$ complex, provided only that $[P]_t \gg K_m$, which is likely (see below). In this case K_m simplifies to k_{for}/k_{doc}, and Equation 4 simplifies to Equation 5:

$$V_{en} = k_{for}[M]_t;$$

$$K_{en} = \frac{K_d k_{for}}{k_{doc}[P]_t}. \quad (5)$$

Under these circumstances V_{en}/K_{en}, which is the slope of a V_{en} vs. K_{en} plot, will be independent of k_{for}, depending only on k_{doc}, K_d, and the protein concentrations $[P]_t$ and $[M]_t$ (Eq. 6):

$$\frac{V_{en}}{K_{en}} = \frac{k_{doc}}{K_d}[P]_t[M]_t. \quad (6)$$

Slight fluctuations in k_{for} from one wild-type culture to another, perhaps due to variations in intracellular ATP levels or rates of ATP synthesis, would yield the behavior seen in Figure 3. The alternative explanation, involving changes in a nonproductive binding affinity, cannot explain covariation in a set of identical wild-type cultures. It follows that kinetic dominance by k_{for} may be a genuine feature of wild-type arabinose periplasmic transport, and the most economical way to interpret Figure 1 is to propose that k_{for} dominance has been preserved in all 17 ABP mutants.

If k_{for} does dominate, Equations 5 and 6 allow important limits to be placed on the involvement of the model's four stepwise parameters K_d, k_{doc}, k_{und}, and k_{for} in the mutant uptake phenotypes. The k_{for}'s of the mutants clearly differ from wild type, since mutants were selected

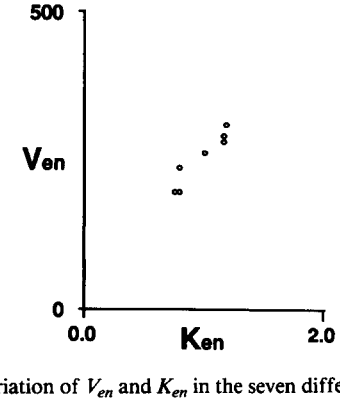

Fig. 3. Covariation of V_{en} and K_{en} in the seven different assays of wild-type arabinose-binding protein, whose average is plotted as the filled circle in Figure 1.

based on their altered V_{en}'s and k_{for} is proportional to V_{en}. Equation 6 implies either that k_{doc}, K_d, and protein expression levels compensate for each other in all mutants (within the precision of the uptake data) or else that they do not change. V_{en}/K_{en} is quite sensitive to protein levels, since it is proportional to both $[P]_t$ and $[M]_t$, yet variation in V_{en}/K_{en} is small among the individual assays of each mutant that are averaged as triplicates in Figure 1. Thus, expression levels probably vary only slightly from culture to culture. If k_{doc} and K_d are to compensate for one another, the k_{doc} for the liganded form of any mutant must decrease (get worse) to the same extent that the K_d decreases (gets better), or else both must increase to the same extent. There is no reason for these two properties to be correlated, and coincidental variation between them in all 18 variants is unlikely. It is more likely that neither k_{doc} nor K_d varies significantly in any mutant.

If docking on-rates are the same for all the mutants, any differences in equilibrium docking affinity $K_s = k_{und}/k_{doc}$ must be due to changes in k_{und}. The k_{und} may indeed vary in some of the ABP mutants in this set. However, because the mutants exhibit the constant V_{en}/K_{en} ratio characteristic of the $k_{for} \gg k_{und}$ condition in every case, their k_{und} values must all remain small compared to k_{for}. The K_s of any particular mutant in the set may be higher or lower than wild type, regardless of its V_{en}. By contrast, K_m is predicted to vary downward from wild type in the down mutants and upward in the up mutants in proportion to changes in their k_{for}'s. Slower transporting mutants would actually have higher steady-state affinities, and vice versa.

The k_{for} can become quite high according to this model (see below), but wild-type *araFGH* does not operate at the maximum possible rate, since three of the ABP point mutants recovered by Kehres and Hogg have twice the forward rate of wild type. The arabinose affinity aspect of this transport system must be biologically relevant. *Escherichia coli* has another arabinose uptake system, the proton-arabinose symporter *araE*, with a lower affinity (K_{en} = 100 μM) but a higher bulk transport capacity (Hogg, 1977). If the primary role of *araFGH* is to detect and scavenge small amounts of arabinose, even the small K_{en} increase that accompanies k_{for} "up" mutations might be deleterious to cells competing for trace amounts of nutrient.

The "severe down," "mild down," "silent," and "up" mutants appear to form four clusters in Figure 1. Such behavior could occur in a k_{for}-dominated process if there were a small number of discrete alternative transition states available in the rate-limiting step, each with a characteristic activation energy (hence k_{for}), and if the primary effect of a set of mutations was to modulate those energies. Single amino acid substitutions might alter only a few intermediates, but might change the rank order of their free energies in such a way that unrelated mutants would end up selecting identical intermediates (and k_{for}'s). The model makes no predictions about transition states, and there are no obvious spatial or chemical similarities among ABP mutants in each group that would account for their k_{for}'s being so similar.

Justification of the model

Available evidence is consistent with the assumptions and qualitative predictions of this model. Stopped-flow measurements of the binding protein–ligand interaction yield on-rates of less than a microsecond (Miller et al., 1983), but the cycle time of a transporting membrane complex in vivo is milliseconds or longer. (The wild-type V_{en} in Table 1 is equivalent to 51,333 molecules per second per cell, and if each cell has 10^3–10^4 transport complexes [see Manson et al., 1985] each complex averages one transport event every 19–195 ms.) Assumption 1, that ligand acquisition by the binding protein is at equilibrium relative to translocation, thus seems justified.

Equation 4b predicts that K_{en} will always be less than or equal to K_d, regardless of what value K_m assumes. K_{en} is indeed less than K_d for all ABP variants in the Kehres and Hogg study, N205V included. If K_{en} is effectively uncoupled from K_m, periplasmic transport systems would be free to acquire higher k_{for} translocation rates (hence high K_m's) without suffering any penalty in terms of overall substrate affinity. In vitro reconstitution of histidine periplasmic transport (Prossnitz et al., 1989) and differential expression of maltose-binding protein in vivo (Manson et al., 1985) suggest that K_m is between 50 μM and 100 μM in each of these systems, which is substantially higher than their K_{en}'s of 1 μM or lower.

Total binding protein concentrations were estimated to be around 1 mM in each of those studies, so although K_m's are high, it is still possible to saturate the histidine and maltose membrane transport complexes with liganded binding proteins at high substrate concentrations. Because there is no reason for either value to differ greatly in the arabinose system, the crucial requirement leading to Equations 5 and 6 that $[P]_t \gg K_m$ is likely to be satisfied.

Assumption 2 that $[PA{:}M]$ is at a steady state and Assumption 3 that V_{max} depends only on k_{for} are the simplest reasonable assumptions at each point and are not considered further here.

Neglecting $[PA{:}M]$ as a part of $[P]_t$ (Assumption 4) is less exact and less general than including it, but the equation that results when $[PA]$ is written as $[P]_t - [P] - [PA{:}M]$ and then substituted into Equation 1 does not have parameters independent of $[A]$ that can be equated to the experimental V_{en} and K_{en}, and because v_0 values predicted by the two approaches agree to within a few percent at plausible concentrations and affinities for ABP and AraG:AraH, this model neglects $[PA{:}M]$ relative to $[P]$ and $[PA]$ as a reasonable first-order feature, so Equations 4 can be used.

Application to other periplasmic transport systems

Interestingly, the *E. coli* ribose periplasmic transport system does not appear to be dominated by k_{for}. The K_{en}'s of the partial down mutant ribose-binding proteins reported by Binnie et al. (1992) are not correlated with their lowered V_{en}'s. Whatever their relative magnitudes are in wild-type ribose uptake, both k_{for} and K_s must be altered in the transport systems containing these mutants. The ribose-binding protein associates with the Trg chemotaxis receptor protein, and Binnie et al. (1992) found that the Trg interaction site partially overlaps the cleft mouth surface. Because the arabinose-binding protein has no known chemotactic role, *araFGH* may have evolved its dominant k_{for} under different selective constraints.

Several mutations have been reported in the binding protein or membrane protein components of the maltose and histidine periplasmic transport systems. The interpretation of such mutants has been largely confined to locating static features such as sites of subunit interaction (Prossnitz et al., 1988; Treptow & Shuman, 1988; Prossnitz, 1991), energy coupling (Gibson et al., 1991; Shyamala et al., 1991), or substrate specificity (Treptow & Shuman, 1985; Speiser & Ames, 1991) to specific regions of particular subunits. At present no conclusions can be drawn about possible limiting kinetic conditions in these or other periplasmic transport systems, for lack of sufficient data.

At present Equations 5 and 6 only pertain to arabinose transport; however, the model presented in Figure 2 and the relationships in Equations 4 should apply to periplasmic transport in general. The model makes many oversimplifications, both in the number of steps it proposes and in the assumptions it makes about them. The forward step represented by a single k_{for} is actually a combination of several processes, and it is unknown whether opening of P and M, hydrolysis of ATP, dissociation, or some other event is the ultimate rate-limiting step in arabinose uptake. It may not be valid to assume that the dissociation of P and A from M is kinetically irrelevant in other systems. The contribution of $[PA{:}M]$ to $[P]_t$ may not be negligible in all cases either. As the dissection of binding protein-dependent transport continues, and mutagenesis, in vitro reconstitution, and other techniques are brought to bear on a variety of systems, the first-order V_{en} and K_{en} relationships described here should be a useful point of departure for more detailed kinetic analysis.

Acknowledgments

I thank Dr. Robert W. Hogg, in whose laboratory I carried out the arabinose transport studies that form the basis of this report. This work was supported by grant DMB9016747 from the National Science Foundation and training grant HL07415 from the National Institutes of Health. It will be submitted to the Graduate School of Case Western Reserve University in partial fulfillment of the requirements for the Ph.D. degree in Molecular Biology and Microbiology. I also thank Alan Binnie for sharing his ribose transport data prior to publication, and Dr. Kenneth Neet for his encouragement and advice on kinetic matters.

References

Ames, G.F.-L. (1986). Bacterial periplasmic transport systems: Structure, mechanism, and evolution. *Annu. Rev. Biochem. 55*, 397–425.

Binnie, R.A., Zhang, H., Mowbray, S., & Hermodsen, M.A. (1992). Functional mapping of the surface of *Escherichia coli* ribose-binding protein: Mutations that affect chemotaxis and transport. *Protein Sci. 1*, 1642–1651.

Fersht, A. (1985). *Enzyme Structure and Function*, 2nd Ed. W.H. Freeman and Company, New York.

Gibson, A.L., Wagner, L.M., Collins, F.S., & Oxender, D.L. (1991). A bacterial system for investigating transport effects of cystic fibrosis-associated mutations. *Science 254*, 109–111.

Hogg, R.W. (1977). L-Arabinose transport and the L-arabinose binding protein. *J. Supramol. Struct. 6*, 411–417.

Kehres, D.G. & Hogg, R.W. (1992). *Escherichia coli* K12 arabinose-binding protein mutants with altered transport properties. *Protein Sci. 1*, 1652–1660.

Manson, M.D., Boos, W., Bassford, P.J., Jr., & Rasmussen, B.A. (1985). Dependence of maltose transport and chemotaxis on the amount of maltose-binding protein. *J. Biol. Chem. 260*, 9727–9733.

Miller, D.M., III, Olson, J.S., Pflugrath, J.W., & Quiocho, F.A. (1983). Rate of ligand binding to periplasmic proteins involved in bacterial transport and chemotaxis. *J. Biol. Chem. 258*, 13665–13672.

Prossnitz, E. (1991). Determination of a region of the HisJ binding protein involved in the recognition of the membrane complex of the histidine transport system of *Salmonella typhimurium*. *J. Biol. Chem. 266*, 9673–9677.

Prossnitz, E., Gee, A., & Ames, G.F.-L. (1989). Reconstitution of the histidine periplasmic transport system in membrane vesicles. *J. Biol. Chem. 264*, 5006–5014.

Prossnitz, E., Nikaido, K., Ulbrich, S.J., & Ames, G.F.-L. (1988). Formaldehyde and photoactivatable cross-linking of the periplasmic binding protein to a membrane component of the histidine transport system of *Salmonella typhimurium*. *J. Biol. Chem. 263*, 17917–17920.

Shyamala, V., Baichwal, V., Beall, E., & Ames, G.F.-L. (1991). Structure-function analysis of the histidine permease and comparison with cystic fibrosis mutations. *J. Biol. Chem. 266*, 18714–18719.

Speiser, D.M. & Ames, G.F.-L. (1991). *Salmonella typhimurium* histidine periplasmic permease mutations that allow transport in the absence of histidine-binding proteins. *J. Bacteriol. 171*, 1444–1451.

Treptow, N.A. & Shuman, H.A. (1985). Genetic evidence for substrate and periplasmic-binding-protein recognition by the MalF and MalG proteins, cytoplasmic membrane components of the *Escherichia coli* maltose transport system. *J. Bacteriol. 163*, 654–660.

Treptow, N.A. & Shuman, H.A. (1988). Allele-specific *malE* mutations that restore interactions between maltose-binding protein and the inner-membrane components of the maltose transport system. *J. Mol. Biol. 202*, 809–822.