Measurement of the Substrate Dissociation Constant of a Solubilized Membrane Carrier

SUBSTRATE STABILIZATION OF OxlT, THE ANION EXCHANGE PROTEIN OF OXALOBACTER FORMIGENES*  

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Although a comprehensive analysis of membrane transport should include measurements of the substrate dissociation constant ($K_D$), this parameter is not accessible by the kinetic tests generally used. Therefore, as in enzymology, it is customary to use the Michaelis constant for transport ($K_M$) as an initial index of substrate-protein interaction, despite that this term reflects the lumped effect of a collection of more fundamental rate and binding constants (1). Moreover, the kinetic analysis of membrane transport has complexities not often found in enzymology, since even in simple cases catalysis of transport requires that during a single turnover the substrate binding site be exposed, sequentially, to both cis and trans compartments. Clearly, it would be useful to have a simple way to assess $K_D$ directly, independent of the effects of transport itself.

We were faced with this theoretical concern as well as certain technical problems in the analysis of OxlT, a newly described bacterial anion exchange carrier of unusual character. OxlT (for oxalate transporter) is found in *Oxalobacter formigenes*, a cell which uses this antiport protein to exchange divalent oxalate for monovalent formate, thereby generating an internally negative membrane potential (2). Although OxlT has been characterized biochemically (2, 3), certain of its properties pose unexpected problems in analyzing function. For example, in its unusual velocity OxlT differs significantly from the norm. Purified OxlT has a turnover number of at least 500/s (3), some 10–100-fold greater than most other secondary transporters, and although this high catalytic efficiency is of special interest, it makes conventional techniques less useful in kinetic studies. As well, it has been difficult to directly assess the role of formate, since its effects on OxlT-mediated exchanges may be secondary to the changes of internal pH caused by passive movement of the highly permeant formic acid.

To address these issues we have exploited recent advances in the technology of membrane protein solubilization and reconstitution (4–6) to develop new methods to analyze substrate-protein interactions in OxlT. Specifically, various substrates were evaluated as they protected OxlT against a thermal inactivation interposed between protein solubilization and functional reconstitution. An analysis of our findings has supported three general conclusions. On the one hand, the concentration dependence of substrate protections could be interpreted by a simple model to estimate the dissociation constant ($K_D$) of the carrier-substrate complex; for OxlT this has been useful in quantitating parameters less confidently estimated by standard kinetic tests. Second, these experiments provided direct evidence of an interaction between OxlT and formate, reinforcing the idea that formate plays a role in a biological setting. Together, these results indicate that appropriately solubilized material retains its natural "kinetic" properties, suggesting that our approach may be of particular value to the study of membrane proteins, such as secondary carriers, which have no associated catalytic activity to mark their functional status in the solubilized state. Finally, this work revealed that combination of the solubilized OxlT with its substrate conferred an unusual degree of thermal stability. We suggest that this in vitro event, driven by substrate binding energy, points to stabilization of protein structure as a normal episode during membrane transport.
**EXPERIMENTAL PROCEDURES**

**Organism, Growth Conditions, and Preparation of Membrane Vesicles**—As described earlier (2), *O. formigenes* strain OxB was grown anaerobically at pH 7 under a CO2 atmosphere at 37 °C. Membrane vesicles were prepared using high pressure lysis at pH 7 in the presence of 50 mM MOPS/K or 100 mM KPi; vesicles were stored at 25 mg of protein/ml at -80 °C.

**Solubilization of OxlT**—Detergent extracts of *O. formigenes* membrane proteins were prepared at pH 7 by solubilization of membranes (1–2 mg of protein/ml) with 1.25% octyl glucoside,1 0.4% acetone/ether washed *Escherichia coli* phospholipid (5), 20 mM MOPS/K, 1 mM dithiothreitol, 0.75 mM phenylmethylsulfonyl fluoride, and with 20% glycerol added as the osmolyte stabilant (5, 6); mock (lipid) extracts were prepared exactly the same way, but without added protein. Solubilized protein (900–700 µg/ml) was obtained as a supernatant after high speed centrifugation (5). These crude extracts, which contained OxlT as about 5–10% of the total protein (3), could be stored at -70 °C for an indefinite period without loss of activity. To dilute the solubilized protein for reconstitution, the crude extract was mixed with an appropriate volume of mock extract so that all tubes contained approximately the same amounts of lipid and detergent.

**Reconstitution of OxlT and Assays of Transport**—We used two protocols to assess OxlT activity. In the more lengthy method (2, 5) 25–50 µg of solubilized protein was mixed with 6 mg of bath-sonicated liposomes in an appropriate buffer (usually 50 mM MOPS/K, 150 mM KCl, and additional detergent (to 1.25%) in a final volume of 1 ml. Proteoliposomes were then formed at 23 °C by 25-fold dilution into a loading buffer, pH 7, containing 50 mM MOPS/K, 1 mM dithiothreitol, and 100 mM of the desired substrate (usually oxalate) as the potassium salt. Proteoliposomes were collected by centrifugation, then washed and resuspended in 300 µl of assay buffer (20 mM MOPS/K, 75 mM K2SO4) to give a suspension of about 20 µg of protein/ml and 13 mg of lipid/ml (2, 5). OxlT exchange activity was estimated by substrate accumulation at 23 °C after 20-fold dilution of proteoliposomes into assay buffer and addition of 0.12 mM [14C]oxalate or 0.1 mM [14C]malonate (2); other details are provided with the individual experiments. For this method of assay, activity is reported with reference to the substrate-accessible space at the steady state. In these latter protocols to assess OxlT activity. In the more lengthy method (2, 5) the half-life of OxlT was extended to about 200 min (Fig. 1, legend), but even low levels of substrate affected a significantly increased stability. For example, in this and four similar experiments, the presence of 30 µM oxalate increased the OxlT half-life by about 3-fold, from its basal value of 4.9 ± 0.6 min to 17.5 ± 0.8 min (means ± S.E.). The same finding was made at other temperatures; for example, in a separate experiment the presence of 30 µM oxalate increased the life-time of OxlT by a factor of 2.8 ± 0.2, as the half-lives of the untreated controls ranged from 1.5 min (46 °C) to about 40 min (23 °C).

Earlier study indicated that OxlT had an unusually high velocity (2), and as might be expected, this made it difficult to use with confidence conventional sampling techniques in a

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1 The abbreviations used are: octyl glucoside, octyl-β-D-glucopyranoside; MOPS, 4-morpholinepropanesulfonic acid.

**Other Assays**—The protein content of membrane vesicles, detergent extracts, and proteoliposomes was measured using a modification (5) of method of Schaffner and Weissmann (7).

**Chemicals**—[14C]Oxalic acid (2.7 µCi/mmol) and [14C]malonic acid (5 µCi/mmol) were purchased from Du Pont-New England Nuclear Corp. Phospholipid was purified from the crude E. coli lipid provided by Avanti Polar Lipids, Inc. Octyl glucoside was from Boehringer Mannheim Corp.

**RESULTS**

**Temperature Sensitivity of Solubilized OxlT**—The experiment of Fig. 1 illustrates our basic observation regarding the temperature sensitivity of OxlT. The solubilized protein was placed at 37 °C, in the absence or presence of oxalate, and thermal stability was assessed by subsequent reconstitution after a 4 °C quench by excess substrate. In this experiment (Fig. 1), OxlT activity in the control (ununtreated) preparation showed simple exponential decay, with a corresponding half-life (τ1/2) of 7 min. The presence of substrate had a striking effect on this thermal lability, and when incubation was carried out in the presence of progressively higher oxalate concentrations, the half-life of OxlT activity increased in parallel. At the highest level of oxalate tested here (1 mM), the half-life of OxlT was extended to about 200 min (Fig. 1, legend), but even low levels of substrate affected a significantly increased stability. For example, in this and four similar experiments, the presence of 30 µM oxalate increased the OxlT half-life by about 3-fold, from its basal value of 4.9 ± 0.6 min to 17.5 ± 0.8 min (means ± S.E.). The same finding was made at other temperatures; for example, in a separate experiment the presence of 30 µM oxalate increased the life-time of OxlT by a factor of 2.8 ± 0.2, as the half-lives of the untreated controls ranged from 1.5 min (46 °C) to about 40 min (23 °C).

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**FIG. 1. Thermal stability of OxlT.** A detergent extract (18 µg protein/ml) was placed at 37 °C in the absence (○) and presence of oxalate at 10 µM (△), 30 µM (■), 100 µM (■), and 1000 µM (■). At the indicated times, aliquots were removed and placed in chilled tubes containing an equal volume of 20 mM oxalate along with the components required for later reconstitution, as described under "Experimental Procedures." After reconstitution, the oxalate-loaded proteoliposomes were tested for residual OxlT activity by the abbreviated assay. OxlT half-lives were 7, 10, 20, and >200 min for samples exposed to 0, 10, 30, and 1000 µM oxalate, respectively. By comparison with the untreated control, Equation (1) (see text) allowed calculation of the corresponding values for a substrate dissociation constant (Kd) of 23, 16, 26, and 36 µM oxalate, respectively.
kinetic analysis of transport (3). Moreover, the presumed in vivo exchange reaction involves movement of oxalate against formate, and tests of formate transport have their own uncertainties due to the high passive permeability of formic acid (2). For these reasons, it seemed sensible to examine more closely the possible significance of the interactions between solubilized OxlT and its substrates (e.g. Fig. 1), with a view to describing these relationships in a quantitative way.

Analysis of OxlT Thermal Stability—Analysis of the effects of substrate on the thermal stability of OxlT followed the general considerations of irreversible inactivation, with simplifications appropriate to this specific example. We have used a scheme in which solubilized OxlT (C) is subject to two events: (a) a thermally driven denaturation (rate constant, \( k_1 \)) that generates an inactive species (C*) and (b) a reversible interaction with substrate (S) (rate constants, \( k_2 \) and \( k_3 \)) to generate a carrier-substrate complex (CS):

\[
\begin{align*}
C + S & \leftrightarrow CS \\
\text{k}_2 & \text{C*} \\
\text{k}_1 & \\
\text{S} & \rightarrow \text{CS}
\end{align*}
\]

This simple scheme incorporates two premises. First, we assume that, as measured here, the transition of C to C* is irreversible, a supposition that appears justified considering the high levels of oxalate used during the quench and subsequent reconstitution. In addition, we assume that the binary complex, CS, is not subject to thermal decay. This is unrealistic in the general case, but seems not far from the mark in the present circumstances. For example, in the accompanying paper (3) no decay of OxlT activity was detected during a 2-h incubation in the presence of 10 mM oxalate, whereas incubation with lower concentrations of oxalate (0.1 and 1 mM) gave the expected losses of activity (cf. Fig. 1). If stability in the presence of 10 mM oxalate is taken to indicate decay of no more than 5% during the sampling interval (120 min), then the rate constants for thermal decay of C and CS would differ by at least 300-fold (half-lives of 4.9 and \( \geq 1440 \) min, respectively). Accordingly, decay of the CS complex is not considered in this analysis. Along with these presumptions, we also assume rapid equilibrium between C, S, and CS, so that the ratio, \([S]/[C]/[CS]\), reflects the dissociation constant \( (K_O) \) for release of substrate from the binary complex. Since OxlT binds and transports substrates with unusual speed (turnover time of 1 to 2 ms), this too seems justified, at least for the time scale of the present work.

With these simplifications, use of standard theory (1) allows one to derive the relationship,

\[
\frac{R}{h} = 1 + \frac{[S]}{K_O}
\]

where \( R \) gives the ratio of the observed rate constants for the C to C* transition found in the absence and presence of a stabilizing ligand (\( k_1 \) and \( k_1' \), respectively); \( R' \) also reflects the \( n \)-fold increase in OxlT lifetime achieved by ligand stabilization.

If the assumptions discussed earlier are appropriate, one expects that Equation 1 will predict comparable values for a substrate dissociation constant as differing degrees of protection are offered by different concentrations of a protecting ligand. Indeed, this appears to be the case. Using the data of Fig. 1, derived \( K_O \) values ranged between 16 and 36 \( \mu M \) as oxalate concentration was varied over a 100-fold range (Fig. 1, legend). One should note, however, that the \( K_O \) derived from this analysis (20 \( \pm 7 \) \( \mu M \) oxalate) is 10-fold lower than the apparent Michaelis constant for transport measured in prior work (\( K_T \) of 0.24 \( mM \) oxalate) (2). Such a difference might arise on theoretical grounds (although it need not), but two technical issues should be addressed before assigning special significance to this discrepancy. First, the earlier (and present) assays of reconstitution employ an internal substrate concentration of 100 \( mM \). Inevitably, there is a residue of external substrate not removed by washing of proteoliposomes and perhaps also from unexpected breakage (2, 5). Because this residue is 30-50 \( \mu M \) for our usual assay conditions (2), external substrate concentrations below about 50 \( \mu M \) have not been tested in assays of transport (2), and interactions at low oxalate levels would have been overlooked. Second, as noted earlier, standard sampling techniques may not have been adequate for the very rapid oxalate self-exchange reaction (2). For these reasons, such kinetic studies may be most useful for their qualitative, not their quantitative, impact.

Identification of Other Substrates of OxlT—The successful measurement of a \( K_O \) for oxalate (above) suggested that this simple approach would be a useful supplement to biochemical studies of OxlT. For this reason, we undertook to identify other OxlT substrates to determine whether they, too, might protect the solubilized carrier. Early work had indicated that among the divalent anions related to oxalate ("OOC-COO"), malonate ("OOC-CH₂-COO") might serve as a substrate for OxlT. This conclusion was based on the finding of a 50% inhibition of oxalate self-exchange when 5 \( mM \) malonate was added to the assay, whereas for the same conditions addition of succinate ("OOC-CH₂-C₃H₅-COO") or oxaloacetate ("OOC-CO-CH₂-COO") had no effect (2).

To extend these preliminary findings, experiments were designed to reveal the corresponding heterologous exchanges by monitoring the transport of \([¹⁴C]oxalate into proteoliposomes loaded with a putative substrate (Fig. 2). As expected, movements of oxalate were unusually rapid, and the self-exchange was complete within about 30 \( s \). And of the alternate divalent substrates, only malonate supported a clear and

![Fig. 2. Malonate is a substrate for OxlT.](image-url)
positive response; for similar conditions oxaloacetate-loaded proteoliposomes gave a response of questionable significance (2% of the oxalate-loaded control), whereas succinate-loaded particles were entirely inactive (data not shown). In further studies of reconstitution using the crude detergent extract, we observed a malonate self-exchange (not shown) and characterized this as having a Michaelis constant of 0.77 mM malonate and a maximal velocity of 21 μmol/min/mg of protein (Fig. 3A); these values suggest that, compared with oxalate, malonate is a substrate of relatively low affinity and low velocity (Ref. 2 and see below). We also verified that at its steady state, the in/out distribution of [13C]malonate was altered in the same way when the external pool was expanded by additions of oxalate or malonate (Fig. 3B). This proved that external oxalate and [13C]malonate had access to the same internal compartment, one whose size corresponded to about 0.25 μl/mg phospholipid or roughly one-fourth of the total internal volume (Ref. 7; cf. Fig. 3, legend).

These experiments (Fig. 3) did not specify whether malonate interacts with OxIT as the divalent or monovalent anion, and to make this determination, we examined the electrical character of oxalate:malonate exchange. For this purpose, oxalate-loaded proteoliposomes were exposed to [13C]oxalate in the absence or presence of the potassium ionophore valinomycin. At the steady state (10 min), a test substrate was added in excess (5 mM), and we noted whether the presence of valinomycin influenced the rate of loss of [13C]oxalate (Table I). In this kind of test, substrate loss by neutral antiport should be unaffected by the ionophore, whereas an electrogenic efflux should be accelerated by the parallel movement of potassium which prevents development of an otherwise rate-limiting membrane potential. These expectations were confirmed by the responses to oxalate and formate, which engage in exchanges that are, respectively, neutral (divalent:divalent, accelerated by valinomycin). Therefore, failure of the ionophore to affect malonate:oxalate antiport (Table I) showed that this latter reaction is the neutral exchange of divalent anions. It is also evident that the experiment of Table I gave no evidence that either oxaloacetate or succinate behaved as substrates of OxIT, in accord with the findings cited earlier.

Having resolved questions regarding alternate divalent substrates of OxIT, we next examined monovalent anions that might substitute for formate. Those tests centered on acetate and propionate as candidates, because proteoliposomes loaded with these anions showed low, but significant, accumulation of oxalate (Ref. 2; see also Fig. 2). For this reason, in the experiment described by Table I we also noted the behavior of [13C]oxalate-loaded proteoliposomes following additions of acetate or propionate. Clearly, failure of acetate or propionate to provoke loss of [13C]oxalate, despite use of valinomycin, shows that these monoanions do not interact directly with OxIT. This suggests that the earlier positive responses (Fig. 2) arose from other factors, the most likely of which is oxalate:hydroxyl exchange (via OxIT) after an internal alkanization caused by outward diffusion of the protonated acids (acetate, propionate). This phenomenon need not be unexpected, since as noted earlier (2), the same biological effect arises whether oxalate moves against formate or hydroxyl.

**Determination of K0 Values for Formate and Malonate—** Collectively, the experiments described here (Figs. 2 and 4 and Table I), and in an earlier report (2), identify three substrates of OxIT: the divalent anions, oxalate and malonate, and the monovalent anion formate. If one extrapolates from the observation that oxalate stabilizes solubilized OxIT (Fig. 1), a similarly positive effect is expected on the part of malonate and formate, but not for the compounds not transported by OxIT (oxaloacetate, succinate, acetate, and propionate). Our final experiments have verified these suppositions (Fig. 4). In this work, using the protocol established earlier, we exposed solubilized OxIT to a range of concentrations of each substrate. The presence of malonate had the expected stabilizing influence, and as malonate levels ranged from 0.5 to 10 mM, the half-life of OxIT was extended from its basal value of 4.5 to about 33 min (Fig. 4A); using these data and

![Fig. 3. Kinetic analysis of malonate self-exchange.](image-url)
was determined the ethanol carrier. At the steady state of oxalate accumulation the presence of
and estimated from standard assays of transport. Although
and formate) Table I summarizes the
at final concentrations of 5 mM; the residual content of [14C]oxalate was determined 1 and 5 min thereafter.

| Added substrate          | [14C]Oxalate remaining | 1 min | 5 min |
|-------------------------|------------------------|-------|-------|
|                         | µmol/mg protein        | -Val | +Val |
| Control (assay buffer)  | 35                     | 35    | 35    |
| Oxaloacetate            | 32                     | 32    | 31    |
| Succinate               | 35                     | 32    | 34    |
| Acetate                 | 30                     | 32    | 29    |
| Propionate              | 35                     | 32    | 31    |
| Malonate                | 23                     | 24    | 15    |
| Formate                 | 19                     | 6     | 15    |
| Oxalate                 | 6                      | 5     | 3     |

Fig. 4. Malonate and formate stabilize solubilized OxlT. A, a detergent extract (7 µg of protein/ml) was placed at 37 °C in the absence and presence of malonate. Decay of OxlT was determined as described in the legend to Fig. 1. OxlT half-lives were 4.5, 7, 12, and 32.5 min, respectively, for samples exposed to 0 (O), 0.5 (□), 1 (△) and 10 mM (○) malonate; by comparison with the untreated control (Equation 1), substrate stabilization gave estimated KD values of 0.9, 1.2, and 1.6 mM malonate, respectively. B, in a similar experiment, decay of OxlT was measured when formate was present at 0 (O), 0.4 (△), 2 (□), and 10 mM (○). The measured half-lives were, respectively, 4, 4.5, 6, and 24 min, with corresponding KD values of 3.2, 4, and 2 mM formate during substrate stabilization.

Table I

**Table I**

**The effect of valinomycin on oxalate-linked exchanges**

Oxalate-loaded proteoliposomes were exposed to [14C]oxalate in the presence of 1 µM valinomycin (Val) or the equivalent amount of the ethanol carrier. At the steady state of oxalate accumulation (35 µmol of oxalate/mg of protein), the indicated compounds were added at final concentrations of 5 mM; the residual content of [14C]oxalate was determined 1 and 5 min thereafter.

**Table II**

**Measurement of substrate interactions for OxlT**

The KD for oxalate (18 ± 3 µM) was from six separate experiments (nine trials), including that of Fig. 1. KD values for malonate and formate were taken from Fig. 4. The KD for oxalate transport was reported in previous studies (2) and that for malonate transport from the experiment described in Fig. 2A. The KD for formate was measured earlier (2) as the concentration of formate required to inhibit the oxalate self-exchange reaction by 50% under conditions presumed (not proven) to minimize development of a membrane potential.

In developing this alternative, we choose to directly examine the detergent-solubilized material. Prior work with UhpT, another bacterial exchange carrier, indicated that the soluble protein would retain its natural substrate binding site (4), and improved biochemical methods (4-6) made it possible to monitor the results of experimental work by a subsequent reconstitution. We had also observed that soluble OxlT has a marked temperature lability (as does UhpT (Ref. 4)) and that low concentrations of substrate protect against thermal decay (Fig. 1). Together, these findings predicted that quantitative analysis of such substrate stabilization could be both feasible and informative. This course of action had the added advantage that heat denaturation is an all-or-none phenomenon (8), so that residual activity reflects a smaller population of fully functional molecules rather than a collection of partially active molecules. This, combined with the ability to quickly reconstitute so as to monitor substrate-accessible space, independent of the rate of transport, made OxlT a particularly favorable example to use in exploring the general idea.

Our experiments provide convincing evidence that this approach is valuable to a description of OxlT. Only authentic substrates stabilize solubilized OxlT, and they do so with an effectiveness (formalized as KD) which parallels the hierarchy of substrate affinities during transport itself (Table II). For malonate and formate there was rough quantitative agreement in these different parameters, yet the KD for oxalate was about 10-fold lower than its apparent KT (Table II). Presently, the origin of this discrepancy is not clear, but because of technical concerns associated with the assay of oxalate self-exchange (see above), the KD for oxalate (~20 µM) should be taken as the appropriate quantitative measure of interactions between OxlT and oxalate, whereas KT should be considered a purely phenomenological constant, without theoretical implication.

Our findings also strengthen the assignment of formate as
a substrate of OxlT. In early work, an evaluation of the transport and effects of formate had been made difficult by the
electrogenic nature of the oxalate-formate exchange and by passive movement of the permute formic acid (2). But OxlT stabilization by formate (Fig. 4B) now gives unequivocal evidence of a direct interaction between OxlT and this mono-
valent anion. In the same way, we reinforce the conclusion that other monovalent anions (acetate, propionate) are not
substrates of OxlT, making it more likely that these anions effect oxalate accumulation indirectly, by virtue of changes of internal pH (cf. Fig. 2 and text).

Advantages and Limitations of the General Approach—Our conclusions concerning OxlT indicate that K_D measurements using the soluble protein accurately reflect the properties of the membrane-bound species. This suggests, in turn, that our approach might have wider application. Measurements of K_D normally require use of high affinity substrates or substantial amounts of the target protein or both. But these criteria are not easily met by most experimental systems, and even when they are, it is not always clear that K_D measurements have biological significance. In studies of LacY, for example, one may conveniently study substrate binding in membrane frag-
ments with the high affinity probe, thiogalactoside (9,10). Yet the transport of this analog recruits a kinetic cycle which differs considerably from that found using lactose, the natural but low affinity substrate (11,12). By contrast, the strategy introduced here is suitable for all substrates, even those of low affinity (cf. Table II), using only crude detergent extracts and under conditions that avoid the electrical or chemical gradients which inevitably complicate a rate analysis (13). For experiments of the sort shown here, the main requirement is that the protein be solubilized in active form, and although this constraint may have been unrealistic in the past, it is becoming less so as new methods are introduced (4-6).

Despite these positive comments, one should not be uncritical of the general method, since OxlT may represent an unusually favorable case study. Perhaps the most important limitation is that such experiments average the responses of all forms of the liganded protein, and this may not always be appropriate. In the absence of ion-motive gradients, many but low affinity substrate

Implications for the Mechanism of Membrane Transport—A pronounced thermal stability arises when OxlT (or UhpT (Ref. 4)) binds its substrates (Fig. 1), and whereas it is not

surprising that ligand binding stabilizes protein structure, this occurs with unusual intensity for these membrane proteins. In the present case, increased stability is attributable to a new configuration adopted after the in vitro transition of C to CS, and it is possible to describe this event in a semi-
quantitative way. For example, saturating substrate (e.g. 10 mM oxalate) led to a decrease by at least 300-fold in the rate constant for OxlT decay (cf. Fig. 1 and accompanying text).

Accordingly, the interaction between OxlT and substrate makes available at least 3.5 kcal/mol of binding energy in support of the C to CS transition. Given that this reflects the in vivo event (see above), it is of considerable interest to expand the argument by proposing that the consequence of substrate binding, increased protein stability, is a normal episode during membrane transport. In fact, this possibility has a clear intuitive appeal. Because membrane transport involves spatial rather than chemical transformations, catal-
ysis would seem to require stabilization of protein structure (CS) rather than, as in enzymology, stabilization (tight bind-
ing) of the reaction intermediate (CS'). For both cases, of course, the overall effect is the same, to increase the lifetime and concentration of a specific transition state complex (CS or CS'), thereby accelerating reaction rate. One cannot yet describe the structural reorganization that accompanies this event during formation of CS, since the minimal required binding energy (3.5 kcal/mol) could be accounted for by a small number of new interactions or by an extended conformational change with a small net difference in structural energy. A challenge for future work will be to apply appropriate physical methods to directly examine the structural cor-
relates of such processes. If the interpretation made here is realistic, a secondary carrier such as OxlT may provide a valuable model system in such efforts.

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7 According to absolute rate theory (19), each 10-fold change in rate constant is accompanied by a free energy change of about 1.4 kcal/mol (57 °C).