BIOCHEMISTRY

 Volume 44, Number 24 June 21, 2005

New Concepts

Ligand−Protein Interaction in Biomembrane Carriers. The Induced Transition Fit of Transport Catalysis†

Martin Klingenberg*

Institute of Physiological Chemistry, University of Munich, Schillerstrasse 44, 80336 Munich, Germany

Received March 24, 2005; Revised Manuscript Received May 9, 2005

ABSTRACT: Carrier-linked transport through biomembranes is treated under the view of catalysis. As in enzymes, substrate−protein interaction yields catalytic energy in overcoming the activation barrier. At variance with enzymes, catalytic energy is concentrated on structural changes of the carrier rather than on the substrate destabilization for facilitating the global protein rearrangements during transport. A transition state is invoked in which the binding site assumes the best fit to the substrate, whereas in the two ground (internal and external) states, the fit is poor. The maximum binding energy released in the transition state provides catalytic energy to enable the large carrier protein transformations associated with transport. This "induced transition fit" (ITF) of carrier catalysis provides a framework of rules, concerning specificity, unidirectional versus exchange type transport, directing inhibitors to the ground state instead of the transition state, and excluding simultaneous chemical and transport catalysis (vectorial group translocation). The possible role of external energy sources (ATP and $\Delta\psi$) in supplementing the catalytic energy is elucidated. The analysis of the structure−function relationship based on new carrier structures may be challenged to account for the workings of the ITF.

Solute transport through biological membranes is an essentially catalytic process analogous to enzymatic reactions (*1*, *2*). Both are facilitated by proteins, which guarantee selectivity and the catalytic acceleration of their respective functions. Great strides have been made in understanding the mechanism of enzyme catalysis at the molecular level, mostly due to the abundant availability of enzyme structures and engineered modifications of discrete residues (see the review in ref *3*). Conversely, understanding of the catalytic and molecular events in transport catalysis is less advanced, although a large often sophisticated body of kinetic studies on solute transport through biomembranes has been built, producing various models for the transport mechanism. With a multitude of probes, important insights into the structure−function relationship of carriers were obtained. Only recently have crystal structures of carriers become known, producing an advanced platform for analyzing translocation mechanisms (*4*−*11*).

The performance of enzymes and carriers is inherently governed by energetic principles, which have fundamental mechanistic implications. Whereas the energetic aspects of enzyme catalysis are quite well understood (*12*, *13*), they have only sporadically been discussed for transport catalysis (*1*). Here we will elaborate the energetic principles of carrier-catalyzed solute transport, partially based on previously published outlines (*2*, *14*). Our treatment introduces the "transition state" as an essential step in transport-linked

† This work has been supported by grants from the Deutsche Forschungsgemeinschaft.

* To whom correspondence should be addressed. E-mail: klingenberg@med.uni-muenchen.de. Telephone: 49-89-218075473. Fax: 49-89-218075003.

10.1021/bi050543r CCC: $30.25
Published on Web 05/25/2005

structural changes of the carrier. At variance with enzymes, the transition strain or destabilization is concentrated in the protein rather than in the substrate, driven by optimization of the substrate fit to the binding site in the transition state. The increased level of interaction provides the energy for the rearrangements in the carrier during transport catalysis. This "induced transition fit" (ITF)[1] mechanism enables rationalization and prediction of a number of transport characteristics; only a few of them can be elucidated here. The ITF can also be expected to be a guideline for understanding the intramolecular events which emerge with the advent of carrier structures.

Catalysis by Enzymes and Carriers

The classical view of enzyme catalysis is based on the transition state destabilization of the substrate (*12*, *13*, *15*, *16*). In the substrate−enzyme complex (Michaelis complex), the substrate does not perfectly fit to the enzyme binding center, which is designed for a best fit of the activated substrate. Thus, the transfer into the transition state is driven by the enhanced binding energy of the strained substrate. Concomitantly, the enzyme may undergo a limited local structural adaptation to optimize the fit between the catalytic residues and the strained substrate. However, it is favorable for catalysis that the changes in the enzyme are minor; i.e., the binding center a priori has a configuration largely complementary to the final transition state of the substrate (*12*).

In some enzymes, the induced fit of substrate binding improves binding by aligning residues with the substrate (*17*). It precedes the formation of the Michaelis complex and increases the specificity of substrate selection. In other cases, induced fit may serve to prepare it for the transition state. In both cases, the changes at the binding center distract energy needed for the substrate distortion in the transition state, thus lowering catalytic efficiency (*12*). Besides increasing specificity, induced fit might have evolved for regulatory purposes, transforming the enzyme from the inactive to active state, which is again detrimental to catalytic efficiency. These considerations of substrate-induced conformation changes will be relevant for understanding substrate-induced carrier conformation changes in transport catalysis.

For approaching catalysis of transport, it is useful to first concentrate on cases in which catalysis is isolated from the superimposed chemical and electrochemical energy inputs. Here, as with enzymes, the interaction of the substrate with the carrier is the principal source of catalytic energy. However, at variance with enzymes, the substrate is not chemically altered but instead translocated across a membrane. Given these contrasting prerequisites, the question of how the interaction of the substrate with the protein translates into efficient transport catalysis arises.

Because of the absence of a chemical reaction, in transport the intervention of a transition state has been largely ignored and the analysis of transport from the viewpoint of catalytic energy balance has received little attention. However, also in transport catalysis, a transition state has to exist, even with ephemeral stability, to provide the catalytic energy in line with catalytic laws. As in enzymes, different interactions of the substrate with the carrier in the primary binding step and in the subsequent transition state remain the cornerstones that facilitate transport. The nature of the transition state in transport will be the key to resolving this issue.

This transition state is not to be confounded with that reported for the ATP hydrolysis site of ABC transporters (*18*). Also, in the analysis of binding energy utilization in ATP-driven cation transport of the Ca^{2+} pump (*1*), the emphasis was on energy from ATP hydrolysis, whereas the catalytic effect on the translocation per se was not addressed. Admittedly, here the aspect of translocation catalysis may be subordinate to the workings of the energy input into the uphill transport.

Single-Binding Center Gated Pore Model

For the following considerations, a simple model of transport will be used which is the obvious homologue to a simple enzyme mechanism. At variance with enzymes, the binding site presents to the substrate two "opposite" aspects depending on the opening of the access paths to either side of the membrane and resulting in different interactions with the substrate. The switch between both states accomplishes the substrate translocation. Various molecular models have been proposed on the basis of elaborate kinetic studies of transport systems (see reviews in refs *19*−*21*) or more speculative grounds (*22*, *23*), but it was with the ADP/ATP carrier of mitochondria that a single-binding center gated pore mechanism (SBGP), less precisely called "alternating access mechanism", was first established on a carrier level, based on side specific inhibitor ligands (*24*−*27*) (Figure 1A). With two different highly effective inhibitors specific for the cytosol or matrix side, the ADP/ATP carrier population could be switched entirely from the "external" to the "internal" state or vice versa, provided that substrate ADP or ATP were present. In this "c to m state" transition, one substrate molecule per ADP/ATP carrier was translocated. The binding of the substrate triggers a coordinated, global conformation change of the transmembrane path by which the entry branch is closed and the opposite branch opened for the exit of the substrate. This alternating access amounts to a gating of the translocation path on either side of the single binding site.

These results showed the critical importance of substrate binding in triggering the transition between the two states and concomitant gating. While the requirement for substrate is not absolute, it is dominant in the obligatory exchange type of transport (solute counter transport), e.g., ATP/ADP carrier or Cl^-/HCO_3^- exchanger. In unidirectional transport, exemplified by the glucose facilitator, gating can occur also without substrate binding. However, here and in other typical transport facilitators, the "return" rate of the empty carrier is lower and thus rate-limiting due to a higher activation barrier without substrate binding. As a result, in an imposed exchange mode, exemplified by the glucose carrier in erythrocytes with sufficient substrate on both sides of the membrane, transport reaches a much higher rate than in the physiological unidirectional mode (*28*).

Induced Transition Fit

In the described SBGP (Figure 1A), the catalytic aspect, i.e., the question of what enables the carrier to translocate a

[1] Abbreviations: ITF, induced transition fit; SBGP, single-binding center gated pore; ABC, ATP binding cassette; AAC, ADP/ATP carrier; $\Delta\psi$, membrane potential.

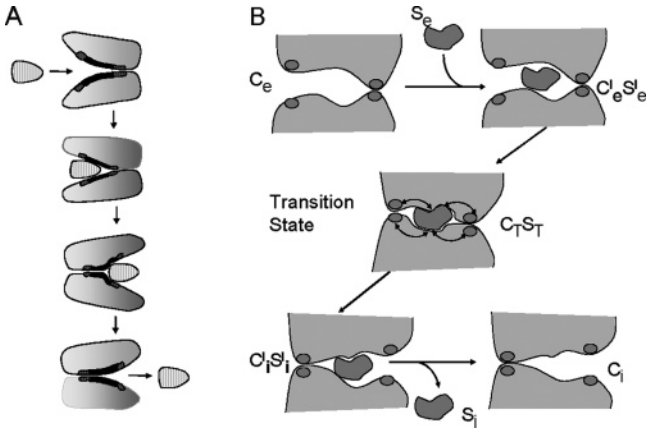

FIGURE 1: Schemes of substrate−carrier interactions in transport catalysis. For a clearer presentation, a large substrate was chosen. (A) Single-binding center gated pore mechanism (SBGP) (from ref *29*). After receiving the substrate from the external side, the binding center switches to the inner face to release the substrate and vice versa. Side specific differences of the substrate interactions with the binding center are emphasized. (B) Induced transition fit. Variable binding fit and the transition state (C_TS_T) are added to the single-binding center gated pore mechanism. A poor fit of the substrate to the binding site in the external (C_e) and internal (C_i) ground states but a perfect fit in the transition state of the carrier (C_TS_T). The gating regions involved in opening and closing of the translocation path on either side of the binding site are highlighted in gray. In the transition state, the forces originating from the binding center to drive the gating are represented by arrows. The binding site offers different aspects to the substrate in the C_e and C_i states. Arrows illustrate the feedback from the substrate−carrier interaction to the gating regions (only part of the transmembrane protein with the translocation path is shown).

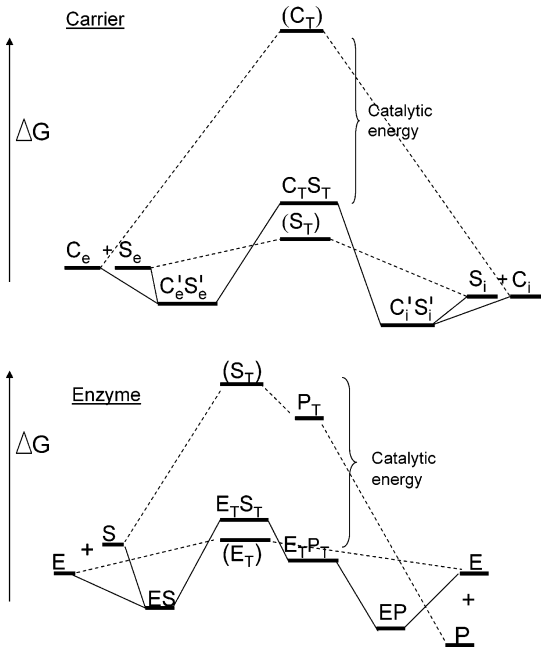

FIGURE 2: Energy diagram of simple models of carrier and enzyme catalysis. In the case of both the carrier and the enzyme, the energy changes are small upon formation of the binding complexes (CS or ES) because of the poor fit but are large going into the transition state, thus largely compensating for the catalytic barrier. In transport catalysis, the activation barrier is largely due to energy needed for the drastic carrier changes; in enzyme catalysis, the barrier is largely caused by the energy needed for the substrate activation. The barrier of uncatalyzed reactions (dashed lines) is leveled by the substrate−proteins interaction energy used as catalytic energy (solid lines).

substrate, is not addressed. The energetic balances of the substrate−carrier interaction and of the carrier transformation are at the core of the catalytic problem. A fundamental treatment of transport catalysis can be obtained by introducing the transition state and a variable substrate−protein interaction, as illustrated in Figure 1B (*2*). As in enzymes, initially the binding site in both the "e" and "i" states should be only partially commensurate to the substrate so that the potential binding energy is not fully exploited. In contrast to enzymes, in the transition state the substrate remains basically unchanged, and instead, the configuration of the binding site is transformed into an optimum fit to the substrate with a concomitant narrowing or closure of both gates. The transition activation is focused on the protein and not on the substrate. The maximum intrinsic binding energy released in the transition state (C_T) is channeled into the machinery of the carrier structure programmed to induce precise global changes which transform the binding site from the external to the internal face. In parallel, the gates are opened and closed at opposite sides of the binding center. In the transition state, the carrier is in an "active" conformation ready to flip into the i or e state ($C'_eS'_e$ or $C'_iS'_i$, respectively).

This substrate-guided flexibility is not to be confounded with free movements in a loose structure; instead, the carrier should follow a precise trajectory of structural changes concomitant with the translocation. Gating and binding optimization in the transition state are inherently connected to these movements. Judging from the few available structures, mutual shifts, rotation, and inclination changes of transmembrane sections may turn out to be a major feature in the rearrangements. The size of these conformation changes will depend among other factors on the size of the substrate.

An energy profile diagram illustrates the implications of the induced transition fit (ITF) for transport catalysis and the fundamental difference of the energy redistribution as compared to enzyme catalysis (Figure 2). In the e or i state without substrate, the carrier is in a stable configuration, corresponding to a comparatively low energy level. When the substrate binds, the limited interaction with the carrier ($C'_eS'_e$) assumes an only slightly changed energy level, which is the balance of energy required for a minor conformation change plus the entropic energy for the substrate immobilization, all paid by the intrinsic binding energy. Since at this stage the binding is imperfect, the complex does not stabilize in an energy trap.

The same holds for a typical simple enzyme catalysis. A fundamental difference is seen in the transition state, where the activation energy barrier is primarily due to global changes in the carrier structure, whereas in enzymes, the barrier is imposed by the substrate deformation in the transition state. These changes require substantial energy input, represented by the high transition state energy level of the unloaded carrier. The barriers are largely leveled by the high intrinsic binding energy released from the intimate substrate−protein interaction in the transition state. The energy difference represents the catalytic energy for transport or product formation. From here, the carrier proceeds to the internal ground state and the enzyme to its original state.

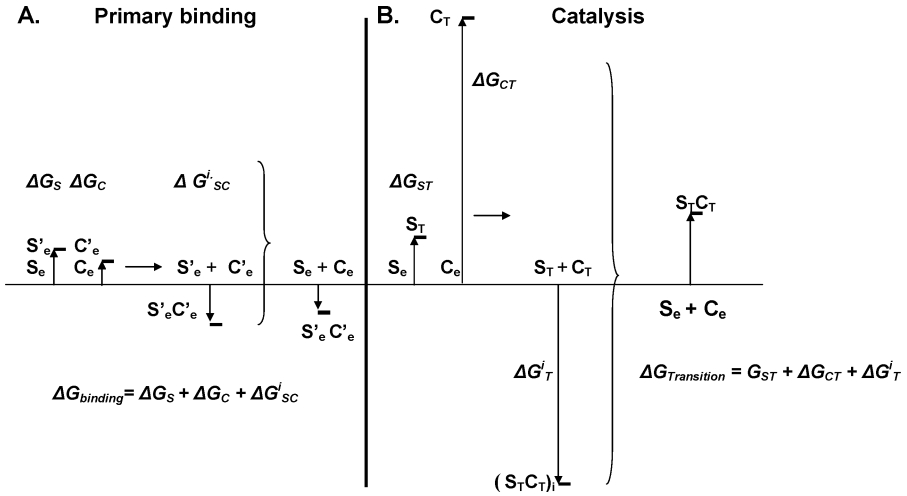

FIGURE 3: Energy changes segregated for the carrier (C) and substrate (S). (A) After primary binding (S'_e), the partial immobilization increases the virtual energy level of the substrate due to a decrease in entropy. The parallel energy change of the carrier is small (C'_e). The intrinsic binding energy ($S'_e + C'_e \rightarrow S'_eC'_e$) release of the performed binding partners surpasses these energy requirements, resulting in a small extrinsic binding energy. (B) Once the reaction proceeds to the transition state, a further entropy loss of the optimally docked substrate increases the virtual energy level of the substrate further ($S_e \rightarrow S_T$), whereas the large rearrangement of the carrier ($C_e \rightarrow C_T$) requires a major virtual energy input. The intrinsic binding energy released in the transition state ($S_T + C_T \rightarrow S_TC_T$) is largely due to the optimum fit, and levels the overall energy requirement ($S_e + C_e \rightarrow S_TC_T$).

An analysis of how the energy requirements are compensated by the intrinsic binding is represented in a diagram which segregates the contributions from substrate and carrier (Figure 3). The primary binding energy is broken down into the parts of first bringing the substrate and carrier into the binding state ($S_e \rightarrow S'_e$, $C_e \rightarrow C'_e$) followed by the "intrinsic" binding ($S'_e + C'_e \rightarrow S'_eC'_e$). For the substrate, the energy requirement ΔG_s is mainly entropic due to restriction of the substrate movement. Because of the poor fit, the substrate still retains some freedom and the energy change is not large. For the carrier, the conformation change with first-stage substrate binding is very small. The limited intrinsic binding energy is able to compensate for these energy requirements. In the transition state, the optimum fit further restricts substrate freedom, requiring more energy ($S_e \rightarrow S_T$), but the major energy demand comes from the virtual transformation of the free carrier to the transition state ($C_e + C_T$). The high intrinsic binding energy released from formation of the transition complex pays for these energy inputs ($C_T + S_T \rightarrow C_TS_T$).

Depending on the energy levels of the carrier−substrate complexes, the carrier will be distributed between ground states $C'_eS'_e$ and $C'_iS'_i$ and transition state C_TS_T. Since the ITF ensures a smooth energy profile, the $C'_eS'_e \leftrightarrow C_TS_T \leftrightarrow C'_iS'_i$ distribution will be highly fluid. In most cases, the population in the transition state may be low due to a somewhat higher energy level. Although the configurations of C_e and C_i are quite different, upon formation of substrate−carrier complexes their energy levels may be similar to ensure maximum catalytic efficiency in an exchange system. Thus, at saturating substrate concentrations, the distribution between C_eS_e and C_iS_i may be even, whereas at nonsaturating concentrations or in the absence of substrate, the carrier will exist as relatively stable and well-segregated C_i and C_e. The distribution between C_i and C_e depends among other factors on the ratio of internal to external substrate concentrations. In an exchange system, the affinity ratio of the two exchanging substrates S_1 and S_2 also will be a factor in this distribution.

As in enzymes, the transfer to the transition state

$$C'_eS'_e \xrightarrow{k_e^{on}} C_TS_T$$

and

$$C'_iS'_i \xrightarrow{k_i^{on}} C_T\,S_T$$

should be rate-limiting. The magnitude of the protein rearrangements will be a major determinant for these rates. The substrate size may be one factor influencing the changes and thus the translocation rate. Indeed, in a comparison of the turnover rates with the molecular weight of the substrate of four different exchange carriers (for Cl^-/HCO^-_3, glucose, lactose, and ADP/ATP), using widely different substrate sizes ranging from ~36 to 500, under conditions of maximum catalytic rates without external force, a linear relation of the logarithmic turnover to the molecular weight (M_r) emerges [$\log k = (0.72 \times 10^{-2})M_r + 6.5$]. The coefficients can be interpreted in terms of rearrangement energy and entropic factors (M. Klingenberg, unpublished results). Since these carriers are located in different membranes, this relation appears to be valid not only for different types of exchangers but also for different environments.

Recent crystal structures of carriers including the AAC are in agreement with the SBGP and discard tandem- or multiple-binding site models (*5*−*7*, *9*−*11*). However, these structures represent only one fixed state, in some cases stabilized by an inhibitory ligand, and thus, the alternating access has not yet been demonstrated. In the structure of a putative glutamate carrier homologue, the substrate seems to be occluded (*9*). It is still inconclusive whether this might represent a transition state. The occluded state of cation pumps generated by arresting the ATP hydrolysis cycle may represent a fixation of the transition state (*30*, *31*) (see below).

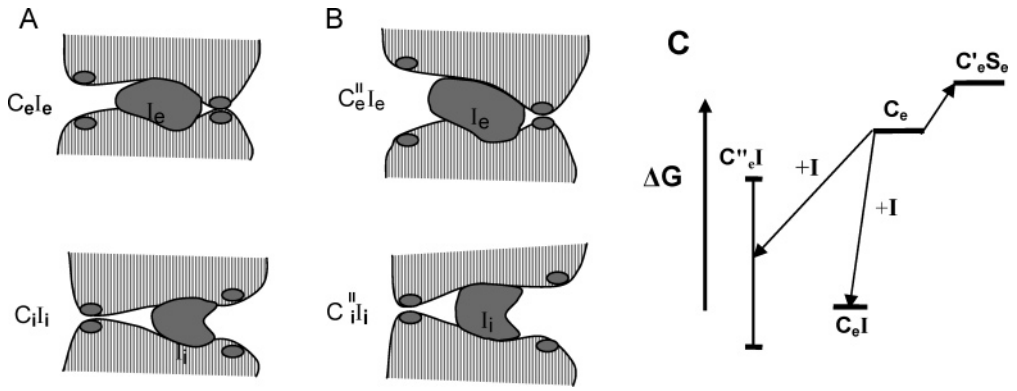

FIGURE 4: Binding of substrate site inhibitors to carriers according to the induced transition fit mechanism. Transport inhibitors bind to the ground states or induce abortive ground states, but do not bind to the transition state. (A) Illustration of the good fit of inhibitors to the binding sites in the ground state. Inhibitors of the e and i states must have different structures. The binding site in the carrier−inhibitor complex has the same configuration as in the ground state depicted in Figure 1B. (B) Large inhibitors may change ground states into a more open configuration, called abortive ground states of the carrier, C''_e and C''_i, a configuration "more distant" from the transition state. (C) Energy profile of inhibitor binding (binding only to the C_e state is shown). The good fit of the ligand to the ground state of the carrier allows the release of the high binding energy, leading to tight binding (for simplicity, any entropic energy requirement is neglected). Larger inhibitors inducing abortive ground states depending on their structure ($C_e + I \rightarrow Ce''I$) can have a range of binding energies, depending on the balance of conformational change and intrinsic binding energy.

Specificity and Inhibitors

The ITF mechanism of carrier catalysis has several important consequences for carrier functions, only some of which can be discussed here. According to the ITF, in the transition state the structure of the carrier-binding center must be complementary to the substrate. The degree of optimization in the transition fit, not the fit to the ground states, primarily determines the substrate specificity of transport. Thus, more efficient catalysis, reflected in high translocation rates, should be related to accentuated substrate specificity. In an exchange system, one of the two exchanging substrates will have a less precise fit and thus be rate-limiting. Again, this straightforward relation only qualifies for the basic catalytic conditions, in the absence of superimposed driving forces.

Given the limited substrate fit to ground states C_e and C_i, certain substrate analogues or related compounds may exist with better complementarities to the ground state. These substances exhibit additional contacts and better "fill" the site than the transported substrate as illustrated in Figure 4A. With a good fit to the ground state, there is no incentive to enter the transition state. Being stuck in an energy trap, these compounds are not transported, and they will be inhibitors of transport as previously exemplified with glucose derivatives for the glucose transporter, depending on the binding affinity (*32*). Thus, at variance with enzymes, in carriers substrate analogues may form "ground state inhibitors" but not "transition state inhibitors". The structure of these compounds should mirror the configuration of the ground state binding sites. Given the different aspects of the e and i ground states, there should exist two groups of potential membrane side specific inhibitors (*32*). The structural differences of the e and i state inhibitors should yield information about the binding site interface in these "opposite" ground states. These compounds, often being larger than the substrates, employ a more extended interface releasing more binding energy.

There are indications that because of the high flexibility, the binding center can be modulated in the e and i ground states under the influence of some inhibitor ligands. Thus, the binding center may adopt extreme configurations which we have called "abortive ground states". As a result of this ligand-induced adaptation, the selectivity can be increased. The "extrinsic" binding affinity can vary depending on the structure of these ligands. The ADP/ATP carrier of mitochondria affords a paradigm of ground state inhibitors with (carboxy) atractylate targeting the e state and bongkrekate the i state (*24*, *33*). They share with ADP and ATP three or four negative charges but, being chemically different, a barely similar molecular shape. Their larger size engages more bonds and may induce the binding site into abortive ground states. With their different structures, they reflect the different binding interfaces in the e and i states.

Under these conditions, the binding sites in the two states provide large "chemical spaces" for the development of ligands and the design of substances which inhibit carriers with high selectivity, thus posing a challenge both for nature and for man. The development of drugs, bactericides, pesticides, or herbicides, for targeting transport systems has to be cognizant of the ITF by taking into account the postulate that substrate site inhibitors of carriers differ from those of enzymes.

Exchange versus Unidirectional Transport and Active Transport

The ITF influences the working conditions of exchange versus unidirectional transport. Exchangers are the transporters par excellence, clearly set apart from channels, since they most consistently fulfill the criteria of carrier-catalyzed transport. Frequently, they meet or approach the facilitating type of transport, whereas in unidirectional transport, the facilitating mode is rare and occurs mostly as active transport driven by superimposed forces.

For the "pure" unidirectional facilitator case, in line with the ITF the return branch of the "empty" carrier is generally rate limiting, lacking catalytic energy from substrate binding to permit adequate overall rates. It follows from the ITF that unidirectional transport is associated with comparatively smaller conformation changes so that the activation energy barrier for the empty hemicycle remains at a sufficiently low

level. The smaller substrate-induced change results in a less precise fit and consequently in lower specificity. Thus, as a rule, substrates with weak interactions and/or small molecular size are preferred by unidirectional facilitators.

How can we integrate external energy supply, driving "uphill" transport into the realms of the ITF? Only a brief glance at this important question is possible here. External energy transfer, by membrane potential or ATP hydrolysis, mainly serves to enable uphill transport against osmotic and electrochemical gradients. We propose that ATP or membrane potential may also serve, additionally or exclusively, to compensate for the lack of binding energy in particular in the substrate-less "empty branch" of the unidirectional transport cycle by supplying catalytic energy to the translocation. The same mechanism by which ATP drives the delivery of the substrate, i.e., by skewing the binding site toward one of the two ground states, may also decrease the barrier inherent to the transition state. Catalytic acceleration is advantageous even if the invested energy is not recovered in a gradient. This external energy-driven catalysis may be dominant in electroneutral ATP-dependent transport as in some ABC transporters. ATP causes drastic conformation changes in the nucleotide binding domains which translate into the reorientation of the substrate sites in the transmembrane domain. In cation pumps, most of the ATP hydrolysis energy is invested into electrochemical gradients. But the transition state exemplified by the "occluded states" of cation binding indicates ATP-driven binding enhancement for catalysis. This contribution may be more accentuated for the electroneutral branch of the exchange cycle, i.e., the K^+ import by the Na^+/K^+ pump, in accordance with a preferred Rb^+ occlusion (*30*). Energy requirements for the conformation changes to drive the cation uphill and to facilitate transport are entwined. Focusing on the exclusive use of the external energy for uphill transport may fail in understanding of the interaction of the cations with the protein.

Vectorial Group Translocation

Originally, the concept of vectorial group translocation seemed to be a deceptively elegant variant of active transport. By integration of a chemical transformation with substrate transport, the energy released from the chemical reaction drives the import. A membrane-embedded enzyme combines the chemical and vectorial reactions so that the substrate enters and the product leaves at opposite sides of the membrane. Mitchell (*34*) elaborated the group translocation and called it a "chemiosmotic" process. According to the ITF mechanism, vectorial group translocation is unfavorable with respect to transport catalysis since chemical and transport catalysis would counteract each other because of opposing requirements for the configuration of the binding site in the transition states. In other terms, the binding energy at a single site would not be able to sufficiently sustain both types of catalytic energies. In fact, vectorial group translocation, once considered to work in several membrane-bound reactions, has not been confirmed in a strict sense for any case. Originally, the PTS system for transport of sugar into bacteria was regarded as vectorial group transfer (see the review in ref *35*). However, in the membrane-bound "IIBC" subunit, where the sugar is transported and phosphorylated, transport and phosphorylation sites are separate (*36*). Interestingly, the phosphate donor is a phosphorylated protein rather than ATP which recruits catalytic energy for the phosphate transfer not from the substrate site but from the protein−protein interface, thus circumventing the opposing energy source requirements for enzymatic and translocation catalysis at the substrate site.

Induced Transition Fit Exemplified in the ADP/ATP Carrier of Mitochondria

The ADP/ATP exchanger (AAC) of mitochondria qualifies with a variety of functions as a paradigm for demonstrating the ITF mechanism of carrier catalysis. Although the exchange of ADP/ATP across the inner mitochondrial membrane is driven by membrane potential ($\Delta\Psi$) by exploiting the charge difference between ATP^{4-} and ADP^{3-}, the AAC also catalyzes electroneutral homoexchanges between ADP or ATP only (*37*). These unproductive modes are important for the autoregulation of the AAC by the ADP/ATP ratios in vivo, as they occupy to a variable degree AAC transport capacity, thus decreasing the productive $\Delta\Psi$-driven heteroexchange. Exclusive homoexchange can be studied in vitro with isolated mitochondria or reconstituted AAC-containing vesicles (*38*, *39*), and thus, the $\Delta\Psi$ influence can be separated and pure transport catalysis studied. The rates of homoexchange and $\Delta\Psi$-driven heteroexchange are similar, attesting to the high efficiency of the transport catalysis in the AAC. The transport of the large and highly charged molecules requires large structural rearrangements in the AAC and a correspondingly high-energy input from substrate binding in line with the ITF. In the AAC, nucleotide−protein interactions have evolved particular strength by exploiting the full charges of "naked" ADP^{3-} or ATP^{4-} without the intervention of Mg^{2+} (*24*). In addition, a high selectivity for adenine reflects a precise fit of the base moiety with numerous bonds. This cumulative high binding energy is necessary to pay for the equally high energy demand of the large structural changes associated with the translocation of large substrates. Thus, the paradox of the low affinity of nucleotide binding and unusually high specificity of transport is accommodated well by the ITF, resulting from a weak initial binding fit and a highly selective and optimum fit in the transition state, respectively. Here the large intrinsic binding energy is consumed by the extensive rearrangements of the AAC.

The transport rates are indicative of the efficiency of energy compensation in the ITF. In view of the large energy components that are involved, the balance between conformational and binding energy is delicate. With this in mind, a comparison of transport rates of the AAC with other nucleotide transporters, having similar structures, underpins the workings of the ITF. As compared to those of carriers in general, the transport rate of the AAC is low at $\sim$15000 μmol min^{-1} (g of protein)$^{-1}$ at 25 °C (*40*) since the extensive rearrangements conditioned by the large substrates prolong the catalytic cycle. Although using nearly identical substrates, the rates of other intracellular, mostly mitochondrial nucleotide transporters are even distinctly lower: between 500 and 900 μmol min^{-1} (g of protein)$^{-1}$ [exchangers for deoxy ADP/ATP (*41*), for GDP/GTP (*42*), for ATP-Mg/P_i (*43*), and for ADP/ATP in peroxisomes (*44*)]. This divergence can be accommodated with the ITF, considering the different intrinsic binding energies; instead of using the fully charged nucleotides as in AAC, the

negative charges are partially neutralized by H^+ or by Mg^{2+} in the alternate nucleotide carriers. The decreased electrostatic interactions provide less catalytic energy, although the transport using similarly sized molecules should be faced with a similarly high activation barrier. Further, these carriers have a lower selectivity for the base moiety than the AAC, reflecting a less perfect substrate complementarity of the binding site. The inability of the highly effective AAC inhibitors, (carboxy)atractylate and bongkrekate, to inhibit any of the alternate nucleotide carrier also testifies to the differences of the nucleotide binding center. In conclusion, with AAC being endowed with an exceptional intrinsic binding energy, its characteristics respond, in line with the ITF, to the high transport demand of ATP supply in the cell. The less perfect catalytic energy balance and resulting lower rates in the alternate nucleotide carriers are in accord with their role in the regulation of mitochondrial and peroxisomal nucleotide homeostasis.

The comparison of nucleotide binding to that of the similarly structured uncoupling protein (UCP) provides a further illustration of the workings of the ITF. In UCP purine nucleotide (G-, A-DP/-TP) are powerful inhibitors of H^+ transport. As in AAC, they bind with their full negative charge and no involvement of Mg^{2+} (*45*). In contrast to that of AAC, the binding affinity is high ($K_D \approx 10^{-7}$, depending on pH) and binding is strictly confined to the c side. Also at variance with AAC, nucleotides induce a higher resistance to proteases, reflecting a more rigid structure in line with their inhibitory function. The higher binding energy in UCP results from the balance between the intrinsic binding and the input for the structural change into the inhibited state. It stands to reason that this is more extensive than the structural changes in the AAC (*46*).

Conclusions

Substrate−protein interaction, elucidated with the novel concept of induced transition fit (ITF) at the core, provides the catalytic energy for the transport. The energetic and molecular constraints generate specific rules for transport characteristics and for substrate−protein complementarities according to whether the ligand is transported or acts as an inhibitor. Although the ITF is most clearly evident in transport facilitators using intrinsic binding energy, it is proposed that in ATP- or $\Delta\Psi$-dependent transport external energy may also be used to maximize the transition fit. The molecular changes implicated in the ITF will be a guideline for analyzing the dynamics of the substrate binding site with the advent of carrier structures.

REFERENCES

1. Jencks, W. P. (1980) The utilization of binding energy in coupled vectorial processes, *Adv. Enzymol. 51*, 57−106.
2. Klingenberg, M. (1991) Mechanistic and energetic aspects of carrier catalysis, in *A Study of Enzymes, Volume II: Mechanism of Enzyme Action* (Kuby, S. A., Ed.) pp 367−388, CRC Press, Boca Raton, FL.
3. Benkovic, S. J., and Hammes-Schiffer, S. (2003) A perspective on enzyme catalysis, *Science 301*, 1196−1202.
4. Locher, K. P. (2004) Structure and mechanism of ABC transporters, *Curr. Opin. Struct. Biol. 14*, 426−431.
5. Lemieux, M. J., Huang, Y., and Wang, D. N. (2004) The structural basis of substrate translocation by the *Escherichia coli* glycerol-3-phosphate transporter: A member of the major facilitator superfamily, *Curr. Opin. Struct. Biol. 14*, 405−412.
6. Abramson, J., Smirnova, I., Kasho, V., Verner, G., Iwata, S., and Kaback, H. R. (2003) The lactose permease of *Escherichia coli*: Overall structure, the sugar-binding site and the alternating access model for transport, *FEBS Lett. 555*, 96−101.
7. Pebay-Peyroula, E., Dahout-Gonzalez, C., Kahn, R., Trezeguet, V., Lauquin, G. J., and Brandolin, G. (2003) Structure of mitochondrial ADP/ATP carrier in complex with carboxyatractyloside, *Nature 426*, 39−44.
8. Abramson, J., Kaback, H. R., and Iwata, S. (2004) Structural comparison of lactose permease and the glycerol-3-phosphate antiporter: Members of the major facilitator superfamily, *Curr. Opin. Struct. Biol. 14*, 413−419.
9. Yernool, D., Boudker, O., Jin, Y., and Gouaux, E. (2004) Structure of a glutamate transporter homologue from *Pyrococcus horikoshii*, *Nature 431*, 811−818.
10. Kunji, E. R., and Harding, M. (2003) Projection structure of the atractyloside-inhibited mitochondrial ADP/ATP carrier of *Saccharomyces cerevisiae*, *J. Biol. Chem. 278*, 36985−36988.
11. Hirai, T., Heymann, J. A., Shi, D., Sarker, R., Maloney, P. C., and Subramaniam, S. (2002) Three-dimensional structure of a bacterial oxalate transporter, *Nat. Struct. Biol. 9*, 597−600.
12. Jencks, W. P. (1975) in *Advances in Enzymology* (Meister, A., Ed.) Vol. 43, pp 219−410, John Wiley & Sons, New York.
13. Fersht, A. R. (1977) *Enzyme structure and mechanism*, Vol. 1, Freeman, San Francisco.
14. Klingenberg, M. (1985) in *Achievements and Perspectives of Mitochondrial Research. Volume I: Bioenergetics* (Quaglieriello, E., Slater, E. C., Palmieri, F., Saccone, C., and Kroon, A. M., Eds.) pp 303−315, Elsevier Science, Amsterdam.
15. Warshel, A., and Levitt, M. (1976) Theoretical studies of enzymic reactions: Dielectric, electrostatic and steric stabilization of the carbonium ion in the reaction of lysozyme, *J. Mol. Biol. 103*, 227−249.
16. Cannon, W. R., and Benkovic, S. J. (1998) Solvation, reorganization energy, and biological catalysis, *J. Biol. Chem. 273*, 26257−26260.
17. Koshland, D. E., Jr. (1960) The active site and enzyme action, *Adv. Enzyme Regul. 22*, 45−97.
18. Chen, J., Sharma, S., Quiocho, F. A., and Davidson, A. L. (2001) Trapping the transition state of an ATP-binding cassette transporter: Evidence for a concerted mechanism of maltose transport, *Proc. Natl. Acad. Sci. U.S.A. 98*, 1525−1530.
19. Stein, W. D. (1986) *Transport and Diffusion across Cell Membranes*, Academic Press, Orlando, FL.
20. Stein, W. D. (1967) *The Movement of Molecules Across Cell Membranes*, Academic Press, New York.
21. West, I. C. (1997) Ligand conduction and the gated-pore mechanism of transmembrane transport, *Biochim. Biophys. Acta 1331*, 213−234.
22. Singer, S. J. (1977) Thermodynamics, the structure of integral membrane proteins and transport, *J. Supramol. Struct. 6*, 313−323.
23. Jardetzky, O. (1966) Simple allosteric model for membrane pumps, *Nature 211*, 969−970.
24. Klingenberg, M. (1976) in *The Enzymes of Biological Membranes: Membrane Transport* (Martonosi, A. N., Ed.) Vol. 3, pp 383−438, Plenum Publishing Corp., New York.
25. Klingenberg, M. (1979) The ADP/ATP shuttle of the mitochondrion, *Trends Biochem. Sci. 4*, 249−252.
26. Klingenberg, M. (1981) Membrane protein oligomeric structure and transport function, *Nature 290*, 449−454.
27. Klingenberg, M. (1985) in *The Enzymes of Biological Membranes* (Martonosi, A. N., Ed.) pp 511−553, Plenum Publishing Corp., New York.
28. LeFevre, P. G., and McGinniss, G. F. (1960) Tracer Exchange vs Net Uptake of Glucose Through Human Red Cell Surface. New Evidence for Carrier-Mediated Diffusion, *J. Gen. Physiol. 44*, 87−103.
29. Klingenberg, M., Aquila, H., Krämer, R., Babel, W., and Feckl, F. (1977) in *Biochemistry of membrane transport, FEBS Symposium No. 42, Zürich 1976* (Semenza, G., and Carafoli, E., Eds.) pp 567−579, Springer-Verlag, Berlin.
30. Glynn, I. M., and Richards, D. E. (1982) Occlusion of rubidium ions by the sodium−potassium pump: Its implications for the mechanism of potassium transport, *J. Physiol. 330*, 17−43.

31. Sorensen, T. L., Moller, J. V., and Nissen, P. (2004) Phosphoryl transfer and calcium ion occlusion in the calcium pump, *Science 304*, 1672−1675.
32. Barnett, J. E., Holman, G. D., Chalkley, R. A., and Munday, K. A. (1975) Evidence for two asymmetric conformational states in the human erythrocyte sugar-transport system, *Biochem. J. 145*, 417−429.
33. Klingenberg, M. (1974) in *Dynamics of Energy-Transducing Membranes* (Ernster, L., Estabrook, R. W., and Slater, E. C., Eds.) pp 511−528, Elsevier Scientific Publishing Co., Amsterdam.
34. Mitchell, P. (1967) in *Advances in Enzymology* (Nord, F. F., Ed.) pp 33−87, Interscience Publishers, New York.
35. Reizer, J., Saier, M. H., Jr., Deutscher, J., Grenier, F., Thompson, J., and Hengstenberg, W. (1988) The phosphoenolpyruvate:sugar phosphotransferase system in gram-positive bacteria: Properties, mechanism, and regulation, *Crit. Rev. Microbiol. 15*, 297−338.
36. Siebold, C., Flukiger, K., Beutler, R., and Erni, B. (2001) Carbohydrate transporters of the bacterial phosphoenolpyruvate: sugar phosphotransferase system (PTS), *FEBS Lett. 504*, 104−111.
37. Klingenberg, M. (1980) The ADP-ATP Translocation in Mitochondria, a Membrane Potential Controlled Transport, *J. Membr. Biol. 56*, 97−105.
38. Krämer, R., and Klingenberg, M. (1980) Reconstitution of adenine nucleotide transport from beef heart mitochondria, *Biochemistry 19*, 556−560.
39. Krämer, R., and Klingenberg, M. (1982) Electrophorectic control of reconstituted adenine nucleotide translocation, *Biochemistry 21*, 1082−1089.
40. Klingenberg, M., Grebe, K., and Appel, M. (1982) Temperature Dependence of ADP/ATP Translocation in Mitochondria, *Eur. J. Biochem. 126*, 263−269.
41. Dolce, V., Fiermonte, G., Runswick, M. J., Palmieri, F., and Walker, J. E. (2001) The human mitochondrial deoxynucleotide carrier and its role in the toxicity of nucleoside antivirals, *Proc. Natl. Acad. Sci. U.S.A. 98*, 2284−2288.
42. Vozza, A., Blanco, E., Palmieri, L., and Palmieri, F. (2004) Identification of the mitochondrial GTP/GDP transporter in *Saccharomyces cerevisiae*, *J. Biol. Chem. 279*, 20850−20857.
43. Fiermonte, G., De Leonardis, F., Todisco, S., Palmieri, L., Lasorsa, F. M., and Palmieri, F. (2004) Identification of the mitochondrial ATP-Mg/P_i transporter. Bacterial expression, reconstitution, functional characterization, and tissue distribution, *J. Biol. Chem. 279*, 30722−30730.
44. Lasorsa, F. M., Scarcia, P., Erdmann, R., Palmieri, F., Rottensteiner, H., and Palmieri, L. (2004) The yeast peroxisomal adenine nucleotide transporter: Characterization of two transport modes and involvement in ΔpH formation across peroxisomal membranes, *Biochem. J. 381*, 581−585.
45. Klingenberg, M., and Huang, S. G. (1999) Structure and function of the uncoupling protein from brown adipose tissue, *Biochim. Biophys. Acta 1415*, 271−296.
46. Klingenberg, M. (1985) Principles of carrier catalysis elucidated by comparing two similar membrane translocators from mitochondria, the ADP/ATP carrier and the uncoupling protein, *Ann. N.Y. Acad. Sci. 456*, 279−288.

BI050543R