Hypothesis

Bacterial flagellar motors and osmoelectric molecular rotation by an axially transmembrane well and turnstile mechanism

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Bacterial ion-driven flagellar motors are the smallest known rotatory mechanical devices, natural or artificial, their overall diameter being only about 25 nm or one millionth of an inch. They are unique in the fields of biology and engineering. This paper develops a possible osmoelectric or local electrokinetic mechanism of molecular rotatory motion in bilayer membranes, which may help to explain how bacterial flagellar motors work, and may incidentally encourage new developments in the bioenergetics and biomechanics of enzyme, osmoenzyme and porter action.

Bioenergetics *Bacterial flagellar motor* *Biomechanics* *Osmoelectric molecular rotation* *Well and turnstile system*

1. INTRODUCTION

Some time ago, it was suggested that bacteria might swim by an electrokinetic type of mechanism, described as self-electrophoresis, operating over the outer surface of the organism and/or its flagella [1,2]. The remarkable discovery of rotatory flagellar motors [3,4], driven by a protonmotive potential difference [5,6], or possibly by a sodium-motive potential difference [7,8], has falsified the part of this suggestion relating to the superficial site of the proposed electrokinetic mechanism of bacterial motility. The practical knowledge about flagellar motors has not, however, diminished the attractiveness of the idea that the kinetic energy of bacterial motility may be derived from the potential energy of certain ions (maintained at different total potentials across bacterial plasma membranes) by means of an electrokinetic type of mechanism, such as that indicated by Glagolev and Skulachev [9]. It therefore seemed to be worthwhile, as described in this paper, to pursue the idea of the electrokinetic mechanism of locomotion further by explicitly formulating the general principles of a local electrokinetic or osmoelectric mechanism for converting osmotic energy to molecular rotational energy. Such a general formulation might possibly help to explain how flagellar motors work, by indicating the general principles for constructing a variety of hypothetical models (and possibly some real artificial models) that could be tested experimentally. It might also be interesting in the more general context of bioenergetics and biomechanics [10,11].

For reasons of descriptive conciseness, attention is focused here on protonic flagellar motors and their hypothetical protonic counterparts. But essentially similar considerations may be applicable to flagellar motors driven by other ion species (such as sodium ions), and to corresponding hypothetical model systems.

2. RATIONALE

2.1. *Salient features of protonic flagellar motor structure and function*

Bacterial flagellar motors have two main structural components [6]. (i) A stator, consisting of a

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set of protein subunits arranged as a circular disk or collar, which lies in approximately the same plane as the plasma membrane, and is connected to it. (ii) A rotor, consisting of a set of protein subunits arranged as a circular disk or plug, which is attached to a central spindle connected to the base of the flagellum. The rotor and central spindle are arranged relative to the neighbouring structures so that they are able to rotate, with the rotor in close apposition to the stator, when a protonic potential difference, Δp, is applied through the motor, but the rotor generally remains locked against rotation by thermal fluctuations when $\Delta p = 0$ [12–14]. It has been suggested that there may be 16 subunits in the stator, and the same number in the rotor ([15] and see [16]). The overall diameter of the motor is about 25 nm, or one millionth of an inch [15].

The rotors of flagellar motors are rotated, on an axis that is normal to the plasma membrane in which the motor is situated, by a current of protons driven through the motor by the total protonic potential difference, Δp, between the outer aqueous medium (P) at positive protonic potential and the cytoplasmic aqueous medium (N) at neutral or negative protonic potential. The direction of rotation may be either clockwise or counterclockwise, and reversals of the direction of rotation normally occur periodically without reversal of polarity of the driving protonic potential across the membrane [12–14]. The predominant direction of rotation and the frequency of reversal depend on the circumstances, and the torque developed is the same for clockwise and counterclockwise rotation [6,12–14,17], as though the direction of rotation were dependent on switching devices associated with an intrinsically eitherway mechanism of torque generation. In this context, it is especially significant that, under circumstances favouring one direction of rotation with the normal polarity of the protonic driving potential, artificial reversal of the driving potential reverses the favoured direction of rotation [14].

2.2. *Elementary osmoelectric principle of ion-driven molecular rotation: axially transmembrane well and turnstile system*

The concept of an ion-driven rotatory motor of molecular dimensions that can run either way is sufficiently novel to make it appropriate to consider, in the broad context of the experimental and theoretical knowledge about flagellar motors, what might be the simplest possible type of transmembrane molecular system, consistent with present day biochemical knowledge, that could use the flow of ions, driven through the membrane by an ionmotive force, to cause eitherway molecular rotation about an axis normal to the plane of the membrane, and parallel to the net direction of the driving force and ion current. Fig.1 illustrates an axially transmembrane well and turnstile system suggested by this approach. The present short paper aims to give a qualitative description of this hypothetical type of system, which is sufficient to define the general osmoelectric or local electrophoretic principle of operation. A more detailed quantitative description will be given elsewhere.

The diagrams in fig.1 show 3 functional polypeptide units plugged through a bilayer membrane, which are supposed to have the following properties. The middle (rotor) component marked R is free to turn on an axis normal to the plane of the bilayer, between the (stator) components marked S_p and S_n on either side of it, which may be anchored to other structures, not shown. The S_p and S_n components of the stator may be identical subunits plugged through the membrane in opposite directions. The rotor R has a proton-binding site (marked B^{m-} in the unprotonated state and BH^{n+} in the protonated state, where $m + n = 1$), located near the surface of the hydrophobic domain of the R polypeptide, which may be protonated and deprotonated only by proton donors and acceptors situated in the hydrophobic domain of the membrane. The stator components S_p and S_n have proton and/or hydroxide conducting systems (shown by the arrows and formally described as proton and hydroxide wells [18,19]) that connect sites marked C_p and C_n at the surface of their hydrophobic domains with the protonically positive and negative aqueous domains P and N on either side of the membrane, so that they can, respectively, donate protons to and accept protons from the proton-binding site on the rotor, when the rotor is at or close to the dead-centre positions shown in fig.1A,B. In this system, the so-called wells in the stator components may be narrow crevices of low dielectric strength that are conducting to a range of hydrophilic ions and hydrophilic solutes, or they may be domains in the stator polypeptide systems

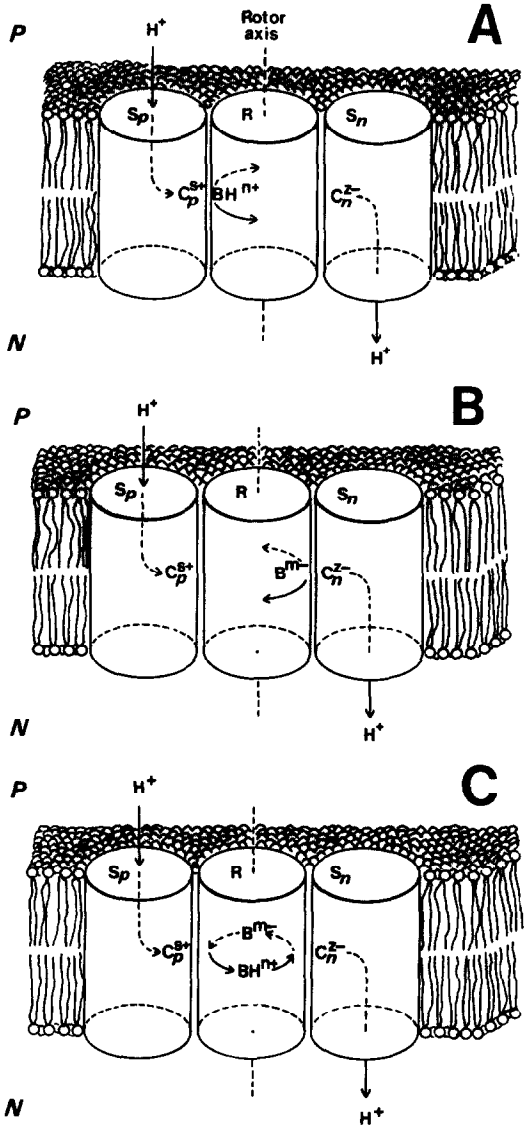

Fig.1. Diagrams of a hypothetical symmetrical well and turnstile molecular motor in a bilayer membrane. The central cylindrical molecule (R) represents the rotor, and the molecules (S_p and S_n) on either side of it represent the stator system. In A and B the rotor molecule R is shown in dead-centre positions, from which it may begin to rotate either way. In C the rotor is shown spinning counterclockwise. Protons are conducted from the aqueous domain *P* to a proton-donating site C_p^{s+} in the molecule S_p; and protons are conducted to the aqueous domain *N* from a proton-accepting site C_n^{z-} in the molecule S_n. A proton-binding site B^{m-} in the rotor molecule R accepts protons from C_p^{s+} at the surface of S_p, and is thereby converted to BH^{n+}, which (after rotation of R) donates protons to C_n^{z-}, and is thereby converted back to B^{m-}. Further explanations are in the text.

that are specifically conducting to H^+ and/or OH^-. The stator sites C_p and C_n at the bottoms of the wells in S_p and S_n, respectively, contain proton accepting and donating groups that give a very high local pH buffering power, and thus partially convert local pH differences to local electric potential differences, according to the principle of the proton trap [19]. For that reason, the equilibration of protons between C_p and the *P* domain, and between C_n and the *N* domain, will partially convert the protonic potential difference across the membrane to an electric potential difference between C_p and C_n, and to a corresponding electric field tending to drive positive charges from C_p to C_n, and negative charges from C_n to C_p. The charges carried by C_p and C_n are denoted by C_p^{s+} and C_n^{z-}, and it is assumed for the moment, for the sake of simplicity, that the plus and minus signs represent net charge (i.e., $z > 0$ and $s > 0$). Thus, when the rotor is at or near the dead-centre positions (fig.1A,B), so that the B site goes to the protonated state BH^{n+} at C_p^{s+}, and to the deprotonated state B^{m-} at C_n^{z-}, provided that BH^{n+} has a net positive charge and B^{m-} has a net negative charge (i.e., $m > 0$ and $n > 0$, or $0 < n < 1$), there will be long-range electric forces tending to drive BH^{n+} from C_p^{s+} to C_n^{z-} and B^{m-} from C_n^{z-} to C_p^{s+}. These forces will be directed through the axis of the rotor in the dead-centre positions, and so they will have no rotatory effect unless thermal fluctuations cause the rotor to move off dead-centre in a clockwise or counterclockwise direction, or unless the rotor is already turning with sufficient angular kinetic energy to enable it to continue clockwise or counterclockwise rotation past the dead-centre points in spite of thermal fluctuations tending to reverse it. Fig.1C illustrates counterclockwise rotation in which the BH^{n+} site is driven from the C_p^{s+} to the C_n^{z-} site via the front semicircular pathway in the diagram. Clockwise rotation occurs similarly when the BH^{n+} site is driven from the C_p^{s+} to the C_n^{z-} site via the back semicircular pathway in the diagram.

Although the distribution of fixed charges on the rotor would make no difference to the overall rotational energy that could theoretically be imparted to the rotor per proton transferred via B from C_p to C_n in the complete rotational cycle, it would affect the kinetic competence of the molecular motor in obvious ways. For example, if the proton-binding group B carried such a net positive

charge in the deprotonated state, or such a net negative charge in the protonated state, that the work required to turn the rotor and move positively charged B from C_n^{z-} to C_p^{s+}, or negatively charged BH from C_p^{s+} to C_n^{z-}, were large compared to the thermal fluctuation energy kT, the rotor would obviously tend to become sequestered in the low-energy position; and if it acquired enough rotational energy to carry it through the low-energy position, forward electric torque generation would operate over only half the cycle, to the detriment of the efficiency of osmokinetic energy conversion. It follows from these considerations that, other things being equal, it would be kinetically favourable for the net charges carried by the deprotonated and protonated proton-binding groups, B^{m-} and BH^{n+}, to be equal and opposite ($m = n = 1/2$).

With the stator components positioned symmetrically on either side of the rotor, so that the C sites and the axis of the rotor would lie in the same plane, and in the absence of any physical difference between the clockwise and counterclockwise semicircular pathways from C_p to C_n, the probability of clockwise and counterclockwise rotation would obviously be the same. However, rotation could be biased in one direction or the other by appropriate asymmetries, either purely spatial, or physical and mechanistic.

Rotational bias could be produced most simply by a spatially asymmetric positioning of the stator components away from the in-line or 180° position, so that the pathway from the C_p to the C_n site was shorter for the proton-binding site of the rotor travelling in one direction of rotation than in the other. The kinetic behaviour of the system would depend on the relative magnitudes of the net charges carried by the protonated and deprotonated forms of the proton-binding site. If, as indicated in fig.2A, the net positive charge carried by BH^{n+} were greater than the net negative charge carried by B^{m-}, rotation would be favoured in the direction for which the pathway from C_p to C_n was shorter, and the field strength and electric force on BH^{n+} was larger; and if, as indicated in fig.2B, the net negative charge carried by B^{m-} was greater than the net positive charge carried by BH^{n+}, rotation would be favoured in the direction for which the pathway from C_n to C_p was shorter, and the field strength and electric force on B^{m-} was larger. But, in the special case that $m = n = 1/2$, rotational bias could not be produced purely by the asymmetric positioning of C_p and C_n. A difference of dielectric strength between the clockwise and counterclockwise pathways from C_p to C_n could have a similar effect to that of spatial asymmetry.

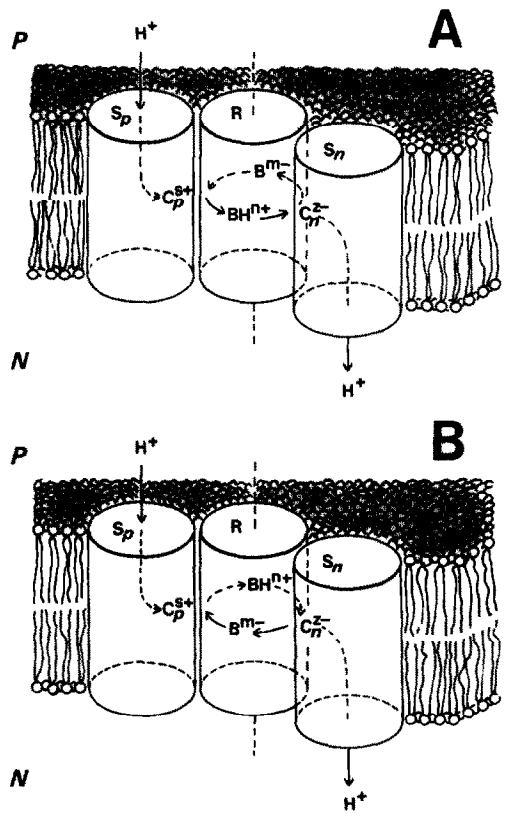

Fig.2. Diagrams of an asymmetric well and turnstile molecular motor that may exhibit a bias towards (A) counterclockwise or (B) clockwise rotation depending on the relative magnitudes of the net charges m and n of the proton-binding centre on the rotor R. Symbol conventions are as in fig.1. Further explanations are in the text.

Rotational bias could alternatively be caused by a physical difference between the clockwise and counterclockwise semicircular pathways from C_p to C_n, such that the proton-binding site on the rotor could pass more readily by one pathway in the protonated state, and/or could pass more readily by the other pathway in the unprotonated state.

2.3. *Oligomeric well and turnstile motor system*

The simple type of osmoelectric motor described

above may be regarded as a primitive protomeric system, containing the essential well and turnstile components out of which more effective oligomeric motors may be built. One of several possible oligomeric systems has been selected for consideration here.

The magnitude and continuity of the torque on the rotor of the protomeric system may be increased by having an oligomeric rotor with several proton-binding sites distributed symmetrically around a central axis. The torque and symmetry of the rotatory forces developed by the stator of the promeric system may also be increased by having an oligomeric stator system with equal numbers of alternate S_p and S_n components with C_p and C_n sites, respectively, distributed symmetrically around the rotor. Fig.3 shows a diagram, in the plane of the membrane, of a motor constructed on this basis, with 16 proton-binding subunits in the rotor and the same number of $S_p + S_n$ subunits in the stator.

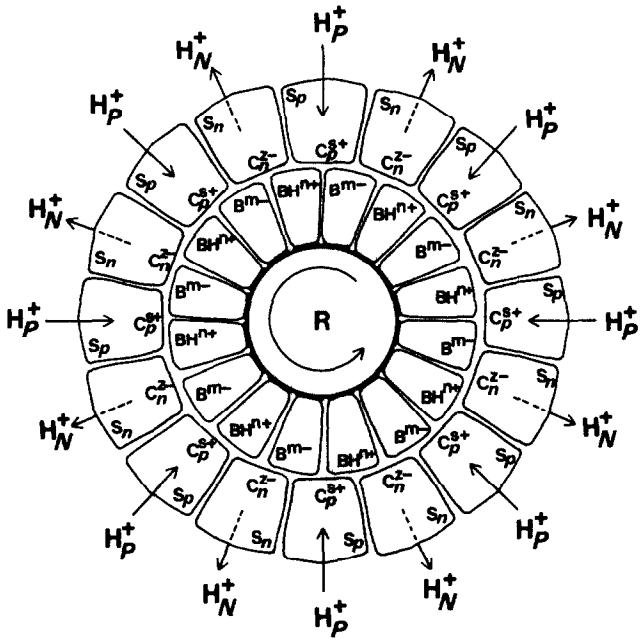

Fig.3. Diagram of an oligomeric proton-driven rotatory motor in the plane of the membrane, viewed down the axis of the rotor R. The symbols H_P^+ and H_N^+ represent protons entering the 8 stator components S_p from the aqueous domain P and leaving the 8 stator components S_n for the aqueous domain N, respectively. Other symbols are as in fig.1, and further explanations are in the text.

The structures of the subunits, in section normal to the membrane, are supposed to be as in the protomeric system, and as in that system, the S_p and S_n components could be identical subunits plugged through the membrane with alternate transmembrane polarities. The eitherway osmoelectric mechanism of torque development would be essentially the same as in the protomeric system of figs. 1 and 2, with the long-range electric forces on the alternately protonated and deprotonated proton-binding sites of the rotor being developed by the electric field between neighbouring C_p and C_n sites on the stator system.

In fig.3, showing counterclockwise rotation, to simplify the appreciation of the mechanism of torque development, the plus and minus signs may be taken to represent the net electric charges on the proton-binding sites B^{m-} and BH^{n+}, and on the stator sites C_p^{s+} and C_n^{z-}, corresponding to the assumption that m, n, z and s are positive numbers. However, by contrast with the protomeric system, the net electric force contributing to torque development on the rotor would continue to be in the forward direction, even when the proton-binding sites carried a net negative charge in the protonated state, or a net positive charge in the deprotonated state (i.e., when $m < 0$ or $n < 0$), because a reverse electric force on the proton-binding sites (that were in the protonated or deprotonated state) on the rotor would be compensated by a stronger forward electric force on the neighbouring proton-binding sites on the rotor (that would be in the deprotonated or protonated state, respectively).

As in the protomeric system, the oligomeric motor could be biased towards counterclockwise or clockwise rotation, either by a purely spatial asymmetry in the positioning of one or more of the C_p or C_n subunits of the stator or corresponding differences of effective dielectric strength (except in the special case that $m = n = 1/2$), or by physical conditions favouring the passage of the proton-binding sites, either in the protonated or in the deprotonated state, between one or more of the pairs of C_p and C_n sites.

For the sake of simplicity in illustrating the general osmoelectric principle, the proton-binding sites B have been represented as monovalent, but this is not a necessary restriction; and there are a number of other spatial, physical and stoichio-

metric ways in which the kind of oligomeric motor system, illustrated in fig.3, might be modified, while retaining the essential osmoelectric principle of conversion of osmotic to rotational energy, which I have sought to describe. It would be inappropriate to attempt a more detailed exposition here, but the hypothetical system outlined above should suffice to indicate the potentialities of this type of conceptual model for assisting in the exploration of the molecular mechanism of bacterial flagellar motors.

It is relevant to note, incidentally, that the osmoelectric type of ion-driven motor mechanism described here, is not restricted to protons, but may be applied to any ion species for which stator and rotor subunits can be constructed with well and ion-trap sites and ion-binding sites of appropriate ion specificity.

3. DISCUSSION

3.1. *Experimental implications of the oligomeric well and turnstile hypothesis of bacterial flagellar motors*

The oligomeric well and turnstile hypothesis of bacterial flagellar motors, developed here from a simple protomeric osmoelectric system, provides a much more conceptually detailed description of the type of system that depends on a coulombic mechanism of torque generation and energy transformation than that previously available [9]; and the kind of physical model described in this paper is comparatively well adapted to experimental verification or falsification. It is especially noteworthy that, although the general type of osmoelectric motor system described here uses a similar electric principle of torque development to that used in the particular model described earlier by Glagolev and Skulachev [9], the oligomeric well and turnstile model developed in the present paper would be expected to exhibit very different properties from those of the earlier model ([9], and see [6,14]).

The characteristics of bacterial flagellar motors that have been the most difficult to explain are: (a) their eitherway rotation without reversal of the polarity of the driving protonic potential difference; (b) their eitherway rotation with the polarity of the driving potential difference reversed; and (c) the reversal of the preferred direction of rotation by reversal of the polarity of the driving potential. These characteristics [6,12–14,17] indicate that the motor is functionally symmetrical with respect to the mechanism by which torque is developed by the flow of protons; and this, in turn, may imply that the motor is physically symmetrical with respect to the torque-producing systems. If, as suggested in this paper, the S_p and S_n components of the oligomeric stator are identical subunits of alternating trans-osmotic-barrier polarity, in the system without rotational bias, the stator will be functionally and structurally symmetrical with respect to the flow of protons. The oligomeric rotor is also supposed to be functionally and structurally symmetrical with respect to the flow of protons. If, as further suggested, rotational bias is caused by spatial or physical asymmetry around the stator, this rotational asymmetry will operate in the reverse sense when the net direction of proton flow through the motor is reversed. Thus, the oligomeric model fulfills the requirements of the special type of reversibility that appears to be characteristic of bacterial flagellar motors. The idea that the rotor and stator each contain only one main type of osmoelectrically functional subunit, and that the asymmetric S subunits are packed in the stator with alternating polarity, is obviously attractive with respect to the structural assembly of the motor, and the possible stepwise assembly of the rotor or stator system may be consistent with observations on the stepwise increase of flagellar torque during synthesis of putative flagellar motor polypeptides [16].

The finding that flagellar torque shows no deuterium isotope effect, and is practically independent of load at constant Δp [12–14] is consistent with the properties expected of the oligomeric osmoelectric system, because the equilibration of protons through the stator subunits S_p and S_n, and between the conduction sites C_p and C_n of the stator and the binding sites B of the rotor, would not be expected to be rate limiting, and at a given Δp and corresponding protonation and electric state of the C_p and C_n sites of the stator and B sites of the rotor, the coulombic interactions responsible for torque development would be independent of load. The observation that the rotor is locked below a threshold Δp, either positive or negative [12–14], suggests that the rotor B sites may not be able to leave the stator C sites unless both are pro-

tonated or both are unprotonated (i.e., only B^{m-} can leave C_n^{z-} and only BH^{n+} can leave C_p^{s+}) and this might be accounted for by purely coulombic interaction energies that are large compared with kT in the $C_p^{s+} - B^{m-}$ and $C_n^{z-} - B^{n+}$ states of the rotor-stator system. The linear dependence of torque on Δp, and the lack of a significant temperature coefficient of the torque at constant Δp [12–14], may be explained on the assumption that the protonic and electric loading of the rotor is independent of Δp above the threshold value, that the electric potential difference between the proton trap systems at C_p and C_n, which would determine the torque, is a constant proportion of Δp, and that this constant of proportionality is temperature-independent, because the same temperature-induced pK shift in the proton traps at C_p and C_n will be mutually compensatory with respect to the difference of protonic and charge loading that determines the electric potential difference between the C_p and C_n sites.

The relatively simple well and turnstile hypothesis developed in this paper appears to have at least as good a chance of explaining the mechanism of flagellar motors as any of the other hypotheses that have been discussed [6,14]. Perhaps its most potentially useful attribute may be that, since it is supposed to involve only two main kinds of osmoelectrically functional subunit with precisely defined properties, one might be encouraged to try to identify the functional properties of these subunits after extraction and reincorporation in bilayer membrane systems.

3.2. *General biochemical implications of ion-driven molecular rotation by coaxial well and turnstile systems*

The machine-like action of catalytic proteins has been receiving increasing attention in recent years (e.g., [20–27]), but the apparent success of the conventional thermodynamic and bioenergetic formalities has not encouraged the development of the corresponding formality of biomechanics. In keeping with this circumstance, the forward process catalysed by enzymes or porters has generally been considered to be so highly damped that it is hardly meaningful to attribute significant net forward momentum or kinetic energy to the masses passing collectively through the closed set of states that constitute the configurational catalytic cycle. The consideration of the mechanics of ion-driven rotatory molecular motors may, perhaps, provide some useful new analogies and insights relevant to the mechanics of enzyme and porter action, and encourage some new initiatives along lines discussed elsewhere [10,11,19,28].

Early theories of carrier-mediated membrane transport that invoked the rotation of carrier molecules, having specific solute-binding groups at their surfaces, had to be abandoned because it was assumed that the axis of rotation of the carrier molecule would be in the plane of the membrane, and this mode of rotation would not be allowed in a carrier protein stabilised in the membrane by means of hydrophilic extremities in apposition to the aqueous phases, and a central hydrophobic domain in apposition to the hydrocarbon domain of the bilayer membrane. However, rotation of protein molecules in bilayer membranes on axes normal to the plane of the membrane is known to occur rather readily. It follows that axially transmembrane well and turnstile systems might give a new lease of life to rotatory theories of carrier-mediated membrane transport.

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