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SCALING THE BARRIER

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[$] Dedicated to J. Christian Schön on the occasion of his 66th birthday

Abstract: *The scaling of activation energy barriers by electron and proton transfer is traditionally overcome in materials chemistry by various strategies, such as by use of extreme temperatures or by quantum tunneling processes. These strategies are not available to biological systems that primarily work under isothermal conditions. Hence, biological transporters have evolved other approaches to transport ions and polar metabolites across membranes, a function of great physiological importance. Energy landscape approaches have proved very useful and have helped quantify the hydrophobic barrier presented to ions by the lipid bilayer membranes. Calculations show that this barrier measures >35 kJ/mol and therefore it is not possible for H^+ ions to scale it unaided. How is this barrier overcome by biological transporters? Nath's two-ion theory of energy coupling and ATP synthesis, first proposed in 2002, and its subsequent development shows that the barrier is considerably lowered by binding of an anion close to the H^+ binding site, thereby enabling rapid, coupled ion transport through biomembranes. A new interpretation in catalytic terms is proposed here, and physical scaling enabling such ion transport is suggested. The aim is to sensitize physicists, chemists, and material scientists to the novel ways by which biological transporters move ions across barriers, and to show the importance of energy landscape approaches in analyzing such membrane transport processes.*

Keywords: *energy barrier crossing, energy landscape, ATP synthase, ion transport*

1. Introduction

It is a pleasure to contribute to this celebration of Christian Schön's 66th birthday. During his career, Christian has covered many different subdisciplines of chemistry and physics, including for example strongly non-linear chemical reactions [1] and the (in)stability of distillation columns [2]. However, most of it has evolved around the energy landscapes on which chemical reactions proceed. For a recent summary see [3]. The most productive path – the optimal path – will typically be the one with the lowest barrier(s) between the reactants and the products. If it is possible to modify the energy landscape, e.g. by changing the composition or the structure of the physical surface on which the reaction typically proceeds, we will have a more efficient reaction; we have developed a catalytic effect. It should also be remembered that the apparent energy landscape depends on the time resolution at which it is observed [4]. The shorter the time scale, the more mountainous the landscape is.

In the present paper we try to achieve the same catalytic result in a biological setting, the driving portion of the ATP forming reaction inside the lipid bilayer membranes of mitochondria [5–7]. The passage of ions, polar molecules, and metabolites through the hydrophobic barrier of the lipid bilayer is a property of great significance to biological systems. Biology has evolved ion channels and membrane-bound transporters in order to surmount such energy barriers [8, 9]. The object is also here to find the path within the given biological structure with the smallest energy barrier.

2. Impossibility of a 1-ion transfer in membrane transporters

The original model for the driving force in generating ATP, proposed by Mitchell in 1966 [10], considers singularly the transfer of H^+ ions from a high concentration (low pH) inside the mitochondrion to a lower concentration (higher pH) in the mitochondrial matrix. Figure 1 illustrates the arrangement in a purified system reconstituted into membranes that synthesize ATP at physiological rates [11]. This transfer of a charged ion through the hydrophobic lipid bilayer membrane is assumed to occur without contribution from any other atomic components. Obviously, that is not a charge-neutral transfer and it will, taken in isolation, build up a potential difference between inside and outside the membrane. Note that there are no oxidation processes regenerating the increased H^+ concentration that could conceivably balance the transport in such a reconstituted system containing only F_OF_1-ATP synthase complexes (Figure 1).

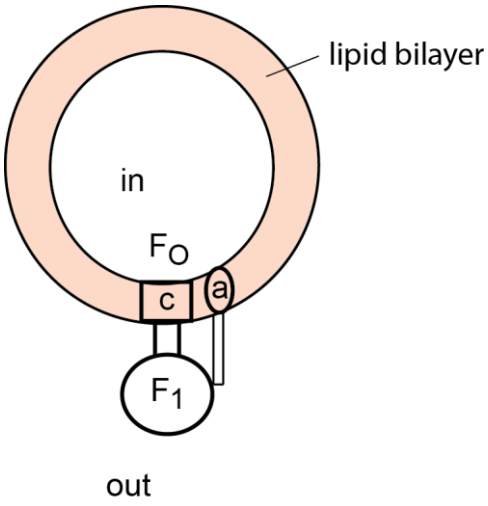

Figure 1. Purified F_OF_1 complex reconstituted into a phospholipid vesicle. This is a clean, single-molecule system without any of the peripheral structures otherwise found in mitochondria. It is considered the gold standard for biochemical work on the subject. A lipid bilayer separates the inside lumen from the outside cell cytosol medium. The inside has a large H^+ and succinate concentration, and the outside has a lower concentration of these ionic species. The F_OF_1 complex, partly embedded in the lipid bilayer, facilitates ATP synthesis driven by the two ion gradients. a and c are components of this structure with the interface containing the path for the transfer of H^+ and succinate ions from inside to outside.

More seriously, this H^+ transport across the membrane is not a channel flow process but is mediated by an ATPase transporter [8, 9, 12]. Unfortunately, this binding-unbinding sequence of microreactions has a free energy barrier of 35 kJ/mol, i.e. roughly 15 kT per particle at room temperature, as shown in Figure 2. As explained by Nath [13, 14], the calculation shows that for various conditions and any reasonable values of the geometric parameters, the barrier height does not change significantly: no H^+ can cross it unaided. If it did, it would be a violation of the first law of thermodynamics in the high-energy region. Thus, a 1-ion transfer is impossible for transpoters in the given biological framework [13, 14].

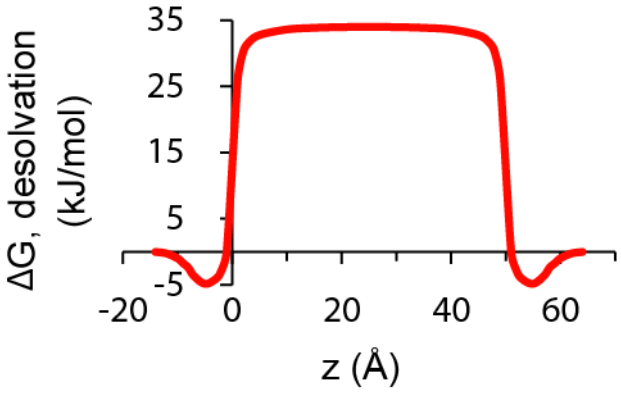

Figure 2. Desolvation Gibbs energy barrier for unaided translocation of H^+ through a biological membrane. The profile is calculated as a function of distance, z, for the transfer of a unit H^+ charge from water into a membrane of thickness L = 50Å with an effective dielectric constant ε_m = 20 [13].

Prohibitively large energy barriers to ion movement through the membrane have been shown for a plethora of transporters by experimental approaches [15–17]. For example, Grewer et al. [15] determined that charge compensation mechanisms are a general feature of ion-coupled transporters. They suggested that "the charge of transported cations must be at least partially compensated for to permit efficient ion translocation, which would otherwise have to overcome large electrostatic barriers of inserting a significant amount of charge into the low dielectric environment of the membrane" [15]. Similar results have been obtained on the energy barriers prevailing in transporters using computational methods. For instance, the group of Roux showed, using continuum electrostatics calculations and free energy simulations, that the dehydration of an ion, as it approaches the channel/transporter, presents a major barrier to the transfer of an ion through it [18]. Despite the above structural, biochemical, and simulation approaches, it has proved challenging to obtain a mechanistic understanding of the transport process. Novel insight into mechanisms was accelerated by the proposal of Nath's torsional mechanism of ATP synthesis and the 2-ion theory of energy coupling [19 and references therein]. This model has been developed in mathematical detail [13]. Quantitative insights into the working of ATPase transporters have subsequently been obtained by a combination of experimental work and theoretical modeling [20, 21]. Innovative energy landscape approaches have also been developed to understand transporter dynamics [22–25]. The interested reader may consult these works for a better understanding of the complex dynamical processes in transporter macromolecules [13, 19–25].

3. Possible resolution

Such an energetic barrier may be overcome in typically 4 different ways: (i) increase the temperature, (ii) quantum mechanically tunneling through the barrier, (iii) wait a very long time for this low probability transfer to occur, and (iv) lower the barrier. Possibility (i) is unrealistic in a biological setting, (ii) is not applicable on this mesoscopic scale, (iii) implies an unrealistically low throughput, although it is seen in some enzymatic processes like the Holliday junction resolution reaction [4], leaving (iv) as the only remaining possibility. Generally, lowering the barrier in a

reaction path and thus increasing the reaction rate is what is achieved by adding a catalyst. A catalyst is not consumed but is returned to its initial state at the end of the reaction. Its contribution is to change the reaction path in state space. Instead of scaling the barrier, another path circumventing the barrier becomes available through the presence of the catalyst.

Such a catalyst could for the present system be an inorganic anion such as Cl^- or an organic anion such as succinate [16, 17, 19, 20], readily available both inside and outside the lipid membrane. Let us call this anion A^-. A major part of the barrier shown in Figure 2 arises from the desolvation of the H^+ ion and electrostatic repulsion between the H^+ and the transporter inside the membrane. By first binding the A^- ion to the transporter in the vicinity of the bound H^+, this repulsion is sharply reduced, and the barrier to charge transport is considerably lowered [13]. On the other side of the membrane the H^+ can detach without strong electrostatic interaction, and as the last step the A^- will be released to the outside of the membrane [20, 21]. This barrier lowering has been experimentally verified [13] as shown in Figure 3. The presence of the A^- has "neutralized" the barrier without changing the initial and final states of the H^+, easing the passage of the hydrophilic H^+ through the strongly hydrophobic membrane. Such a 2-ion transfer process will do the job and does not violate thermodynamic law.

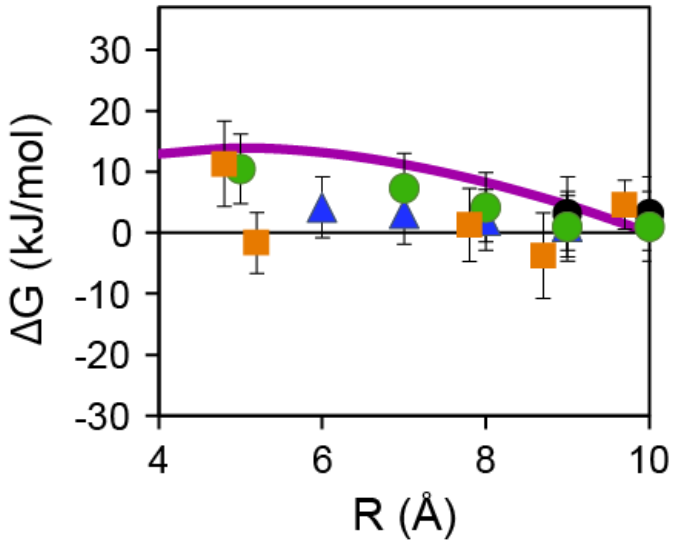

Figure 3. Comparison with experimental data (individual points) of the calculated (solid line) Gibbs energy landscape for ion pairs upon their transfer from water into a membrane transporter. The ΔG is calculated by the Kirkwood-Tanford-Warshel electrostatic theory as a function of the ion pair interionic distance, R, for an effective dielectric constant $\varepsilon_m = 20$ and the hydrated radius of the ions a = 2Å. The 4 experimental data sets (orange squares, blue triangles, green circles, black circles) are for long-range ion pairs, dicarboxylic acids in water, ion pairs in hemoglobin, lysozyme, and cytochrome c, and heme groups, and surface charges in cytochrome c, respectively. For more details, see [13].

4. Scaling

Taking λ to be the distance between the bound negative and positive charges and λ_D the Debye length in the aqueous electrolyte at the entrance of the membrane transporter, we have $\lambda < \lambda_D$ when this coupling between the two ions is effective. (On the other hand, for ion channels $\lambda \geq \lambda_D$). If they are further apart, the surrounding material, primarily water, will effectively shield the two charges from one another and we would have no barrier-reducing effect. Thus we can scale the effectiveness of the catalytic effect of the negative ion by the Debye length.

Essentially, one can say that transporters have evolved a way to function by an ion-coupled mode of transport. If the electrical double layer of the bound proton and anion interact, then a local coupling takes place inside the transporter access pathways. The above scaling then ensures that a mechanical effect takes place by such coupling. The scaling therefore explains the functioning of various transporters and mechano-enzymes. Energy landscape approaches [13, 22–25] are especially appropriate and useful to quantify barriers to ion transfer across biomembranes and determine the extent of their lowering by various strategies.

5. Conclusions

It has been shown here that the presence of an energy barrier, conservatively calculated by electrostatic theories to measure at least 35 kJ/mol, places strong constraints on ion transport through membrane-bound biological transporters. The barrier is shown to be circumvented and scaled by the participation of a catalyst, typically an organic weak acid anion in ATPase transporters or an inorganic anion such as chloride in other transporters. The effect of such coupled ion transfer may be made quantitative by scaling the ion-ion separation with respect to the Debye length in the aqueous electrolyte.

This way of looking at ion transport across biomembranes emphasizes that energy landscape methods are important tools to quantify barrier heights and to analyze ion transport mechanisms in biology. It is most relevant to extend Christian Schön's detailed energy landscape calculations to a biological setting. An interesting difference will be the non-rigidity of the biological systems, i.e. the structure in which the reactions take place can deform to a considerable extent and will fluctuate during normal operation. This will add a whole new dimension to Christian Schön's usual rigid surfaces.

Another topic for future work on biological membrane transport is to extend the landscape methods used for large charged ions by including quantum effects. A general Marcus theory of electron transfer already exists [26, 27], which needs to be adapted or perhaps extended to analyze electron-coupled proton transfer processes that are of central importance to biology.

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