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BRIEF OPINION ARTICLE

Is myelin a mitochondrion?

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It has been hypothesized that myelin acts like a mitochondrion, generating ATP across the membranes of its sheath. By calculating the proton motive force across the myelin membrane based on known values for the pH and membrane potential of the oligodendrocyte, we find that insufficient energy could be harvested from proton flow across the myelin membrane to synthesize ATP. In fact, if the respiratory chain were present in the myelin membrane, then the ATP synthase would function in reverse, hydrolyzing rather than synthesizing ATP. This calculation places the hypothesis of an energy-producing role for myelin in considerable doubt.

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INTRODUCTION

The main method of ATP production in the central nervous system is oxidative phosphorylation, which is carried out by billions of specialized organelles, mitochondria. Mitochondria harvest the energy available in their transmembrane proton gradient to form ATP, which is then exported from the mitochondria for use by energy demanding processes in the cell. It has recently been suggested that myelin—the capacitance-reducing sheath surrounding axons—can carry out the same ATP-producing process.[1] We assess the plausibility of this idea, after outlining both the known mechanism of ATP synthesis in mitochondria and the proposed mechanism of ATP synthesis in myelin.

ATP Generation by Mitochondria

Mitochondria are composed of two compartments: an inner mitochondrial matrix bounded by an inner membrane, and an intermembrane space between the inner and outer membranes (Figure 1A). Within the matrix, the citric acid cycle produces NADH and $FADH_2$, which are passed to the respiratory chain in the inner membrane. Chain complexes catalyze NADH and $FADH_2$ oxidation and O_2 reduction, and the energy released from these reactions is used to pump protons from the matrix to the intermembrane space. This makes the intermembrane space acidic (pH 6.9), leaving the matrix alkaline (pH 7.8) and negatively charged (-200 mV).[2,3]

This electrochemical gradient creates a proton motive force that powers the ATP synthase. Both the electrical and concentration gradients drive protons from the intermembrane space into the matrix. As they flow down this gradient through the F_O segment of the ATP synthase, the energy released is used to power ADP phosphorylation by the F_1 segment of the ATP synthase. One ATP molecule is generated for every 2.7 protons that enter the matrix this way.[4] The ATP is transported out of the mitochondrial matrix in exchange for a cytoplasmic ADP, and an extra proton is cotransported into the mitochondrial matrix with a P_i molecule (it is critical to keep the ratio of ATP concentration to ADP concentration in the mitochondrial matrix low, and the level of P_i high, so that ATP production rather than hydrolysis is favored).

ATP Generation by Myelin: The Hypothesis

Based on evidence from biochemical assays, western blot analysis, and immunocytochemistry, Panfoli's group have put forward the hypothesis that myelin is able to consume oxygen and produce ATP through the operation of an ATP synthase driven by a proton gradient across the membranes of the sheath.[1,5,6] They suggest that mitochondrial fusion with the myelin membrane during formation of the sheath leads to ectopic expression of the respiratory chain complexes and F_1F_O-ATP synthase in the myelin membranes.[6] The respiratory complexes are proposed to pump protons *into* the cytoplasmic space of the myelin sheath, generating a proton motive force that powers the *extracellular* production of ATP via the ectopically expressed ATP synthase (with the F_1 segment facing outward; Figure 1B). ATP is then proposed to be passed from extracellular compartment to extracellular compartment, through a series of gap junctions, until it reaches the axon (although it is unclear how gap junctions—which normally allow diffusion between adjacent intracellular compartments—could mediate ATP movement between the extracellular spaces of myelin, and unclear how ATP would enter the axon). Ravera *et al*[1] suggest that ATP generated in this way is a significant fraction of all the ATP generated in the brain. They also argue that the multilamellar structure of myelin is due to a need for a large area for ATP generation, rather than being due to a need to reduce axonal capacitance.

One potential problem with this arrangement is the possibility that cytochrome *c* expressed on the inner surface of the plasma membrane could be released into the cytoplasm where it would trigger caspase-mediated apoptosis of the oligodendrocytes (reviewed in Jiang and Wang[7]). This is not normally a danger for mitochondrial cytochrome *c*, because it is confined to the mitochondrial intermembrane space by the outer mitochondrial membrane. Furthermore, whether axons, in fact, require metabolic

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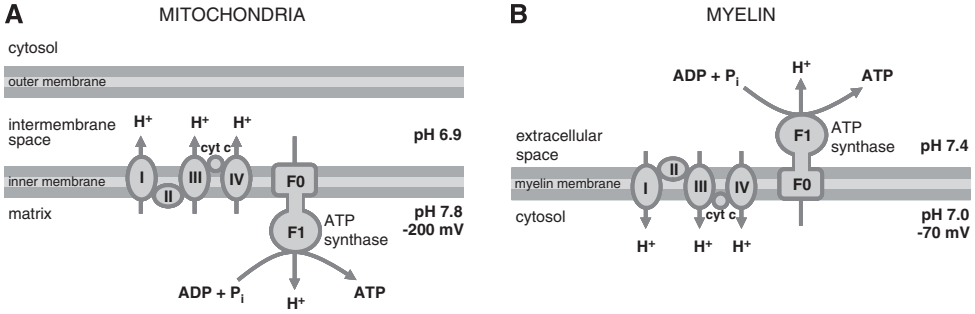

Figure 1. Powering the ATP synthase. (**A**) In mitochondria, respiratory chain complexes (I to IV) pump protons across the inner membrane, making the intermembrane space acidic and leaving the matrix alkaline and negatively charged. This electrochemical gradient creates a proton motive force that powers ATP synthesis in the matrix by the F_1F_O-ATP synthase. (**B**) Ravera *et al*[1,5] have suggested that the respiratory chain complexes and F_1F_O-ATP synthase are ectopically expressed in the myelin membrane. The chain is proposed to pump protons into the cytosol, and their passive outward flow through the ATP synthase would power extracellular ATP production. However, the pH and electrical gradients across myelin membranes make this hypothesis implausible (see text).

support from ensheathing oligodendrocytes is debated. Several lines of evidence suggest that myelinating oligodendrocytes provide energetic substrates, such as lactate, to the axons they ensheath, and that this trophic support is essential for axonal integrity.[8,9] In contrast, Harris and Attwell[10] have calculated that the glucose supply at nodes of Ranvier and the density of axonal mitochondria could make the need for glial metabolic support of central nervous system axons unnecessary, although it is possible that peripheral nervous system axons, with significantly longer internodes, do require such support.

Although it has been suggested that mitochondria routinely contaminate purified myelin preparations, which would provide an alternative explanation for the presence of mitochondrial proteins in myelin,[11,12] we can evaluate the theory put forward by Ravera *et al*[1] on the basis of the requirements it dictates. First, energetic substrates—in particular, NADH and $FADH_2$, the electron donors in oxidative phosphorylation—must be provided extracellularly. Their production (either by the citric acid cycle or glycolysis) must therefore be either extracellular between the sheets of myelin or intracellular with a mechanism for their export. Second, there must be sufficient provision of ADP and P_i to the extracellular space. Third, there must be sufficient clearing of ATP from the extracellular space. Whether any of these critical requirements are met *in vivo* is unknown. However, two features of the oligodendrocyte that are known—its pH and membrane potential—allow us to assess perhaps the most critical prerequisite for the hypothesis: that enough proton motive force can be produced across the myelin membrane to generate ATP. This analysis is presented below in two stages: first, we assess whether protons will tend to move through the ATP synthase in the correct direction to provide energy for ATP synthesis, and then we calculate whether the energy thus produced is sufficient to generate ATP.

METHODS AND RESULTS

For the F_1F_O-ATP synthase to generate ATP, protons must flow from a state of high potential energy on the side of the membrane expressing the F_O segment to a state of low potential energy on the side of the membrane expressing the F_1 segment (where ATP is generated). Both electrical and concentration gradients contribute to the potential energy for proton flow across the membrane. Calculating the change in Gibbs-free energy for proton flow (ΔG_p) tells us whether protons will spontaneously flow across the membrane in the direction that is required for ATP generation.

In the mitochondrion, the F_1 segment of the ATP synthase is in the matrix, so, to synthesize ATP, protons must flow from the intermembrane space to the matrix. Both electrical and chemical gradients across the inner membrane are large and in the right direction, resulting in a negative ΔG_p (Table 1A). This indicates that protons will spontaneously flow across the membrane in the direction required for ATP generation.

In the oligodendrocyte, the F_1 segment of the ATP synthase is proposed to be in the extracellular space, so protons must flow from the intracellular to the extracellular space. With an intracellular pH of 7.0 (ref. 13) and a standard extracellular pH of 7.4, the concentration gradient is in the right direction. However, oligodendrocytes have a resting membrane potential of around -70 mV,[14] implying that the electrical gradient is in the wrong direction. These combine to give a positive ΔG_p (Table 1B; the reversal potential for H^+ is -24 mV, more positive than the resting potential), meaning that protons would not tend to flow across the membrane in the right direction (in fact, passively, they would flow in the opposite direction, into the cell) and cannot provide energy for ATP synthesis. In this scenario, the ATP synthase would operate in reverse, hydrolyzing ATP rather than phosphorylating ADP, while H^+ ions enter the cell. Thus, if myelin expressed respiratory chain proteins in the manner suggested by Morelli *et al*,[6] ATP would be broken down, not generated.

Ectopic expression of the F_1F_O-ATP synthase with the F_1 segment on the extracellular side of the membrane has been proposed for several cells (reviewed in Champagne *et al*[15], Panfoli *et al*[16], and Chi and Pizzo[17]), often based on the observation that the F_1 segment can be antibody-labeled without permeabilizing the membrane (for example, in rat hepatocytes[18]). In myelin, however, it is theoretically possible that the ATP synthase and complex proteins could face the other way, so that the F_1 segment is expressed intracellularly rather than extracellularly. This arrangement would require a different account of how the proteins come to be expressed in the myelin membrane (it could not be by mitochondrial fusion), but perhaps eases some of the difficulties with the original theory, for example, substrate supply and the danger of cytochrome *c*-triggered apoptosis. Most importantly, this arrangement would now require the flow of protons to be from the extacellular to the intracellular space, resulting in a negative ΔG_p and therefore a tendency for passive proton flow in the correct direction (Table 1C).

Thus, for mitochondria, and for myelin with the F_1 segment of the ATP synthase oriented intracellularly, protons would tend to flow in the right direction for ATP generation. But, for ATP to actually be generated, the energy released by this proton flow must overcome the positive ΔG required to phosphorylate ADP (ΔG_{ATP}), which is set by the ratio of substrate (ADP and P_i) to product (ATP) present. Knowing the cytoplasmic and matrix

Table 1. Calculating proton motive force

(A)			
Mitochondria			
Matrix pH (F_1 location)	7.8		Porcelli *et al*[2]
Intermembrane space (IMS) pH	6.9		Porcelli *et al*[2]
Matrix potential	−200	mV	Nicholls and Ferguson[3]
Proton motive force	255	mV	
ΔG for H^+ flow from IMS to matrix, ΔG_p	−24,642	J/mol	
[ATP]/[ADP] ratio in matrix	10		Nicholls and Ferguson[3]
$[P_i]$ in matrix	10	mmol/L	Nicholls and Ferguson[3]
ΔG for ATP synthesis, ΔG_{ATP}	47,500	J/mol	
Net ΔG for flow of 2.7 protons and ATP synthesis ($\Delta G_{ATP} + 2.7x\ \Delta G_P$)	−19,015	J/mol	
Minimum number of H^+ to overcome ΔG_{ATP}	1.93	protons	
(B)			
Myelin with F_1 facing extracellularly			
Intracellular pH	7.0		Boussouf and Gaillard[13]
Extracellular pH (F_1 location)	7.4		
Intracellular potential	−70	mV	Bakiri *et al*[14]
Proton motive force	−45.4	mV	
ΔG for H^+ flow from intracellular to extracellular, ΔG_p	4,380	J/mol	
(C)			
Myelin with F_1 facing intracellularly			
Intracellular pH (F_1 location)	7.0		Boussouf and Gaillard[13]
Extracellular pH	7.4		
Intracellular potential	−70	mV	Bakiri *et al*[14]
Proton motive force	45.4	mV	
ΔG for H^+ flow from extracellular to intracellular, ΔG_p	−4,380	J/mol	
[ATP]/[ADP] ratio in cytoplasm	1,000		Nicholls and Ferguson[3]
$[P_i]$ in cytoplasm	10	mmol/L	Nicholls and Ferguson[3]
ΔG for ATP synthesis, ΔG_{ATP}	60,000	J/mol	
Net ΔG for flow of 2.7 protons and ATP synthesis ($\Delta G_{ATP} + 2.7 \times \Delta G_P$)	48,174	J/mol	
Minimum number of H^+ to overcome ΔG_{ATP}	13.7	protons	

(A) Parameter values for calculating the proton motive force across the inner mitochondrial membrane and the Gibbs-free energy for ATP synthesis in mitochondria. (B) Parameter values for calculating the proton motive force across the myelin membrane from the extracellular to intracellular space. (C) Parameter values for calculating the proton motive force across the myelin membrane from the intracellular to extracellular space, and the Gibbs-free energy for ATP synthesis in myelin.

concentrations of ADP, ATP, and P_i,[3] we can evaluate ΔG_{ATP} in mitochondria and myelin (Tables 1A and 1C). For mitochondria, the energy provided by the proton motive force is sufficient to overcome the energy required for ATP synthesis if >1.93 protons per ATP flow through the ATP synthase. In fact, $8/3 = 2.7$ protons enter per ATP formed (since movement of 8 H^+ is required to turn the F_O part of the synthase through one rotation which generates 3 ATP molecules[4]). Consequently, ATP synthesis occurs (since $\Delta G_{ATP} + 2.7 \times \Delta G_p < 0$; Table 1A). For the same proton flow (2.7 protons/ATP) across the myelin membrane, on the other hand, even with the F_1 segment of the ATP synthase facing intracellularly so that protons would tend to passively flow in the right direction, the proton motive force is not large enough to provide sufficient energy for ATP synthesis (since $\Delta G_{ATP} + 2.7 \times \Delta G_p > 0$; Table 1C). In this arrangement, as with the F_1 segment facing extracellularly, therefore, the ATP synthase would run backwards, hydrolyzing instead of generating ATP and pumping H^+ out of the cell. It would theoretically be possible for the ATP synthase to generate ATP using the same proton motive force if the energy provided by at least 14 protons entering the cytoplasm could be harvested for the synthesis reaction. However, this is far more than the 2.7 protons per ATP molecule normally used[4] and, even if not mechanistically impossible, indicates that powering ATP synthesis by the proton motive force across the myelin membrane would, at the very least, be extremely inefficient.

DISCUSSION

We have assessed the plausibility of an ATP-generating function for myelin at the energetic level. It does not seem possible that enough energy could be provided by the electrochemical gradient for protons across the myelin membrane—no matter which direction the ATP synthase is oriented in—to power the synthesis of ATP. In fact, if the ATP synthase were present in the myelin membrane, then it would function in reverse, hydrolyzing instead of synthesizing ATP.

This analysis is based on known values for the mature oligodendrocyte's resting potential and pH. It is possible that these values, measured at the soma (for the resting potential[14]) and in the absence of proper myelin wraps (for the pH[13]), are drastically different from those of the *in vivo* sheath. It is not clear whether Ravera *et al*[1,5] envisage that ATP is generated within the compact portions of the sheath (where the water content of the intracellular space is almost negligible and discussion of ion concentrations is of dubious significance) or within the inner and outer tongues or Schmidt-Lanterman incisures of the myelin (where pockets of cytoplasm are present). In the latter case, if such cytoplasmic pockets are much lower in pH and more depolarized than the oligodendrocyte cell body, it is theoretically possible that enough proton motive force could be generated across the myelin membrane to synthesize ATP in the manner suggested by Ravera *et al.*[1,5] Experimentally, however, evidence for this is completely lacking: voltage and pH differences between the intracellular

cytoplasmic pockets and the extracellular spaces of myelin have not been measured (and there is no obvious reason, at least for the outer myelin tongue which is well connected to the soma, why such differences should exist). Demonstrating an anomalously positive voltage and acid pH intracellularly in myelin would be essential for the hypothesis that myelin can carry out proton motive force-powered ATP synthesis since, to be taken seriously, its most basic prerequisite—a sufficient proton motive force—must be met. Based on what is currently known about myelin, however, this seems unlikely.

We therefore suggest that previous data indicating the presence of the F_1F_O-ATP synthase in myelin membranes reflect contamination of myelin preparations by mitochondrial membrane (possibly from mitochondria located in the Schmidt-Lanterman incisures or inner/outer myelin tongues[11]) and nonspecificity of antibody labeling. Furthermore, apparent extracellular synthesis of ATP may, in fact, reflect regulated release of intracellular ATP (reviewed in Corriden and Insel[19]). Although here we have focused on the hypothesis of ATP production by myelin, the same line of reasoning is worth considering for similar theories of extracellular ATP production in other cells, e.g., endothelial cells[20] and hepatocytes.[18]

Finally, it is possible that the F_1F_O-ATP synthase is expressed ectopically in myelin, but not in an energy-producing capacity. In hepatocytes, for example, ectopic F_1F_O-ATP synthase expression has a role in regulating the extracellular ADP concentration by hydrolyzing—but not by synthesizing—ATP.[21]

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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