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.

Keywords: ATP synthase; energy; oligodendrocyte; oxidative phosphorylation

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 FADH2, which are passed to the respiratory chain in the inner membrane. Chain complexes catalyze NADH and FADH2 oxidation and O2 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 (ΔpH ~ 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 F0 segment of the ATP synthase, the energy released is used to power ADP phosphorylation by the F1 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 Pi molecule (it is critical to keep the ratio of ATP concentration to ADP concentration in the mitochondrial matrix low, and the level of Pi 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.1,3,5,6 They suggest that mitochondrial fusion with the myelin membrane during formation of the sheath leads to ectopic expression of the respiratory chain complexes and F1F0-ATP synthase in the myelin membranes.5 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 F1 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 al7 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 Wang5). 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...
For the F₁Fₒ-ATP synthase to generate ATP, protons must flow from a state of high potential energy on the side of the membrane expressing the Fₒ segment to a state of low potential energy on the side of the membrane expressing the F₁ 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 (ΔGₚ) tells us whether protons will spontaneously flow across the membrane in the direction that is required for ATP generation.

In the mitochondrion, the F₁ 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 ΔGₚ. Table 1A). This indicates that protons will spontaneously flow across the membrane in the direction required for ATP generation.

In the oligodendrocyte, the F₁ 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 ΔGₚ (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.,² ATP would be broken down, not generated.

Ectopic expression of the F₅Fₒ-ATP synthase with the F₁ 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 Pizzo¹⁷), often based on the observation that the F₁ segment can be antibody-labeled without permeabilizing the membrane (for example, in rat hepatocytes¹⁸). In myelin, however, it is theoretically possible that the ATP synthase and complex proteins could face the other way, so that the F₁ 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 extracellular to the intracellular space, resulting in a negative ΔGₚ, and therefore a tendency for passive proton flow in the correct direction (Table 1C).

Thus, for mitochondria, and for myelin with the F₁ 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 (ΔGₚₐₜ), which is set by the ratio of substrate (ADP and P) to product (ATP) present. Knowing the cytoplasmic and matrix
required to turn the FO part of the synthase through one rotation on the other hand, even with the F1 segment of the ATP synthase facing intracellularly so that protons would tend to passively overcome the energy required for ATP synthesis if the energy provided by the proton motive force is sufficient to power the synthesis by the ATP synthase to generate ATP using the same proton motive force 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) and in the absence of proper myelin wraps (for the pH), are drastically different from those of the in vivo sheath. It is not clear whether Rava et al. 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 Rava et al. Experiments, however, evidence for this is completely lacking: voltage and pH differences between the intracellular and extracellular space are not drastic enough to provide the necessary protons (Table 1A) to power ATP synthase in this manner.
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 F₁F₀-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) and nonspecificity of antibody labeling. 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 and hepatocytes.

Finally, it is possible that the F₁F₀-ATP synthase is expressed ectopically in myelin, but not in an energy-producing capacity. In hepatocytes, for example, ectopic F₁F₀-ATP synthase expression has a role in regulating the extracellular ATP concentration by hydrolyzingly—but not by synthesizing—ATP.

**DISCLOSURE/CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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