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Journal
Current Opinion in Electrochemistry, 29

ISSN
2451-9103

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Publication Date
2021-10-01

DOI
10.1016/j.coelec.2021.100742

Current Opinion in Electrochemistry 29:100742
DOI:10.1016/j.coelec.2021.100742

Electrochemical energy for living systems

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Abstract

Plastoquinone and ubiquinone play essential roles in the electron transport chains of chloroplasts and mitochondria by coupling electron transport to the transfer of protons across membranes. The energy of the resulting proton gradient is used to synthesize adenosine triphosphate (ATP) the energy currency of all life. How did quinones first become involved in this process? We have detected several quinone compounds in carbonaceous meteorites that can transport protons in a liposome model system. It is possible that such compounds were available to transport protons in primitive versions of electron transport in early life.

Key words: Electron transport, plastoquinone, ubiquinone, proton gradient, chemiosmosis, carbonaceous meteorites

1. Introduction

Electrochemical reactions are fundamental sources of energy for all life on Earth. The reactions supply energy for most bacterial species and for two specialized membranous structures called chloroplasts in plants and mitochondria in the cells of organisms that use oxygen. Chloroplasts, the organelles that perform photosynthesis, contain the green pigment chlorophyll as a component of their membranes. Photosynthesis begins with an electrochemical reaction when electrons are released from the electronic structure of chlorophyll molecules that enter an excited state when they absorb photons. The electrons travel down an electron transport chain embedded in the membranes in a series of reactions that later provide reducing power for the capture of atmospheric carbon dioxide and synthesis of reduced carbon compounds. Electrons are immediately replaced on the chlorophyll by another system that strips them from water and releases oxygen into the atmosphere. The oxygen and reduced carbon compounds are then used as an energy source by aerobic life which recombines the electrons stored in reduced carbon compounds with oxygen. The energy that is released is in turn used to synthesize ATP, the primary energy currency of all life.

2. Life's electrochemistry: Chemiosmosis

Sixty years ago, Peter Mitchell published a hypothesis in which he invented a new term: chemiosmosis [1]. The aim of the hypothesis was to explain how ATP can be synthesized by chloroplasts and mitochondria. We now know that two of the components of electron transport chains are plastoquinone in chloroplasts and ubiquinone in mitochondria. These quinones are small mobile molecules that can accept electrons on one side of the membrane and transfer them to a receptor on the other side. When quinones are reduced by negatively charged electrons, they must also accept hydrogen ions (protons) from water, but the receptor on the other side of the membrane does not need the hydrogen ions so they are released. This process produces a proton gradient across the membrane (Figure 1). The evidence is unequivocal: proton transport has been measured experimentally in both mitochondria and chloroplasts as well as the proton gradient that is produced [2, 3].

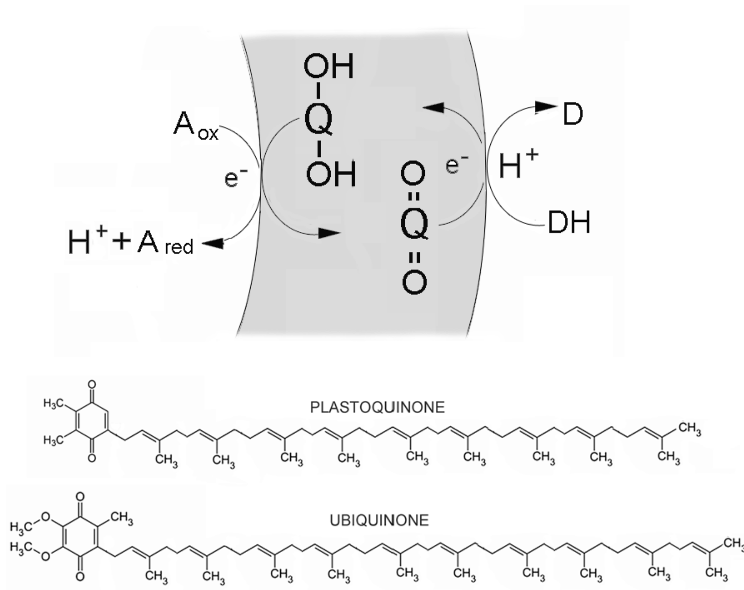


Figure 1. Role of quinones in coupling electron transport to proton transport across bilayer membranes. An external donor source of reducing power (DH) transfers both electrons and protons to a quinone (Q) which diffuses to the other side of the membrane. The electrons reduce an oxidant (A_{ox}) such as an iron compound that does not accept protons in the reaction, and the protons are then released into the interior of the compartment. The structures of plastoquinone and ubiquinone which perform this function in chloroplasts and mitochondria are shown below.

3. Coupling electrochemical reactions of quinones to ATP synthesis

If ATP in an aqueous solution is allowed to be hydrolyzed by an enzymatic catalyst such as ATPase, the reaction reaches equilibrium when the ratio of ATP and ADP concentrations approaches ~1:475. Mitchell proposed that an ATPase embedded in chloroplast and mitochondrial membranes uses the energy of ATP hydrolysis to pump protons across the membrane. Mitchell's brilliant revelation was that if a proton gradient was somehow produced across the membrane by an ATPase pump, according to thermodynamic principles the ATPase could run in reverse and become an ATP synthase. If the proton gradient is produced by an electron transport chain that pumps protons outward, as in mitochondria, the protons of the gradient would leak back through the ATPase and drive the reaction toward ATP synthesis.

Although there was initial skepticism about chemiosmosis, by 1975 sufficient evidence had accumulated to convince the scientific community that it was correct and Mitchell was awarded the Nobel Prize in 1978 [4]. Paul Boyer and John Walker shared a second Nobel Prize in 1997 for establishing the structure of the ATP synthase and the process by which it synthesized ATP [5, 6].

4. Quinones, electrons and protons

From the discussion so far, it is clear that quinones play a primary role in pumping protons across chloroplast and mitochondrial membranes by a process that is coupled to electron transport, but how did quinones begin to play this role in the earliest forms of life? To address this question we need to consider sources of organic compounds on the early Earth before life began. There are just two possibilities that are not mutually exclusive. Either they were synthesized by geochemical or atmospheric reactions, or they were delivered as intact organic compounds. It is important to understand is that *all* of the biogenic elements -- carbon, hydrogen, oxygen, nitrogen, phosphorus and sulfur (CHONPS) -- now present on Earth were delivered during the planet forming process called accretion. These elements were originally components of dust particles in the molecular cloud from which the sun and planets of the solar system formed 4.57 billion years ago. Some of the carbon was present as carbon dioxide mixed with water ice on the surface of the particles, along with thousands of organic carbon compounds including amino acids [7].

We know this for a fact because organic compounds are still being delivered to the Earth on dust particles and carbonaceous meteorites. In September 1969 a bolide exploded in the skies over Murchison, Australia, and meteoritic fragments fell over a five square mile area. Over 100 kilograms were collected by townspeople and scientists who rushed to the scene. One of the stones was analyzed by researchers at NASA Ames and found to contain multiple organic compounds including amino acids and monocarboxylic acids [8]. During the next 50 years hundreds of studies reported that other compounds relevant to life were present in the Murchison meteorite. Figure 2 shows a summary of the main classes.

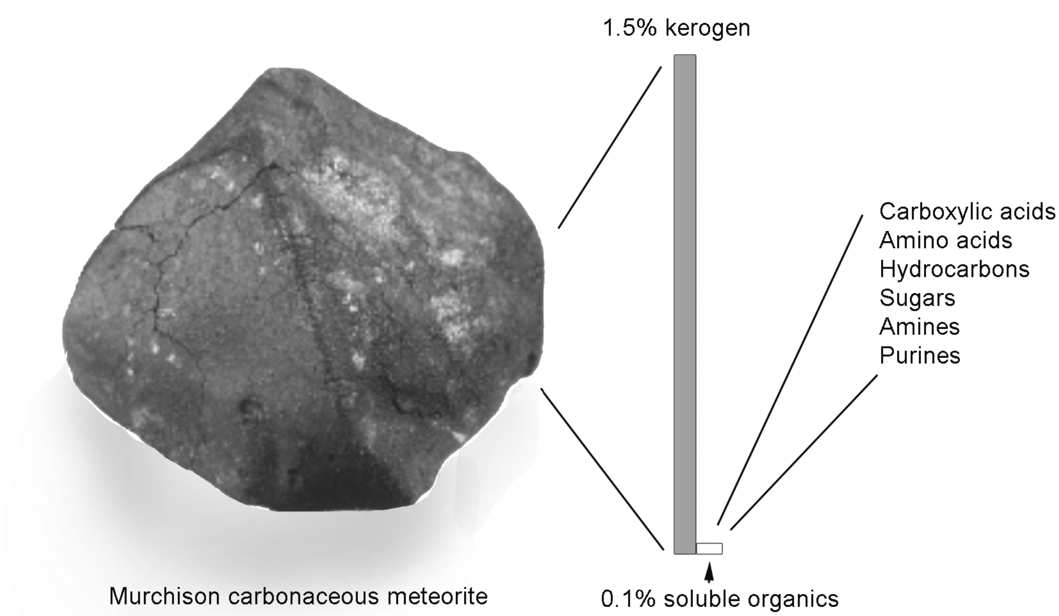


Figure 2. Organic compounds in the Murchison meteorite

The organic carbon content of the Murchison and dozens of other carbonaceous meteorites is a unique guide to the kinds of organic compounds available on the early Earth to take part in the physical and chemical processes leading to the origin of life.

5. The electrochemistry of membrane compartments

With this information in hand, we can now consider how simple electrochemical reactions can generate proton gradients across membranes. Cells are the units of all life today and it is reasonable to assume that life began when self-assembled membranous compartments encapsulated polymers capable of reproducing themselves. Although it is not yet understood how this could have happened, we do know that some form of energy would be required to drive the process. One possible source are the transmembrane proton gradients still being used by life today. Years ago, we observed that a large pH gradient could be generated in a liposome preparation containing an oxidant with a reducing agent present in the external medium [9]. The encapsulated oxidant was ferricyanide and the external reducing agent was ascorbic acid. When an electron carrier called phenazine methosulfate (PMS) was added, it picked up electrons from the ascorbic acid and protons from water, then diffused across the membrane where it was oxidized by the ferricyanide and the protons were released. The magnitude of a pH gradient was measured by a fluorescent dye called 9-aminoacridine which permitted us to watch the internal pH decrease by several pH units in a few seconds (Figure 3A). If we then added nigericin, an antibiotic that allowed proton gradients to equilibrate across the membrane, or Triton X-100, a detergent that caused the lipid bilayer membranes to become leaky to all ions, the gradients rapidly decayed.

We revisited this reaction in 2019 but this time asking whether quinones could have been among the organic compounds present on the early Earth before life began [10]. The project was taken on by Daniel Milshteyn, a brilliant young researcher who was finishing up his undergraduate degree in Biomolecular Engineering on our campus. Daniel extracted the organics of the Murchison and other carbonaceous meteorites using a solvent system that dissolves non-polar compounds such as alkanes, monocarboxylic acids and polycyclic aromatic compounds like anthracene, fluoranthene and pyrene. He prepared liposomes composed of phospholipids mixed with a small amount of the meteorite extract and used ubiquinone as a positive control. The liposomes contained ferricyanide and the redox potential was initialized by adding ascorbic, a reducing agent, to the solution.

A typical result is shown in Figure 3B (ubiquinone) and 3C (Murchison extract). It is clear that ubiquinone can serve as an electron carrier to produce a pH gradient across liposome membranes, but there was also something present in the Murchison extract that served the same purpose. The magnitude of the gradients could be calculated from the amount of quenching of fluorescence. The ΔpH was 2.7 units with PMS or ubiquinone, and 1.9 with the extract. However, there was a slow decay of the gradient caused by the Murchison extract, presumably because some of the components disturbed the lipid membrane and made it more permeable to protons. Therefore we purified the extract by two-dimensional thin layer chromatography and isolated a fluorescent fraction that was likely to contain any quinones present. Figure 3D shows the result. The ΔpH increased to 2.0 and the pH gradient no longer decayed.

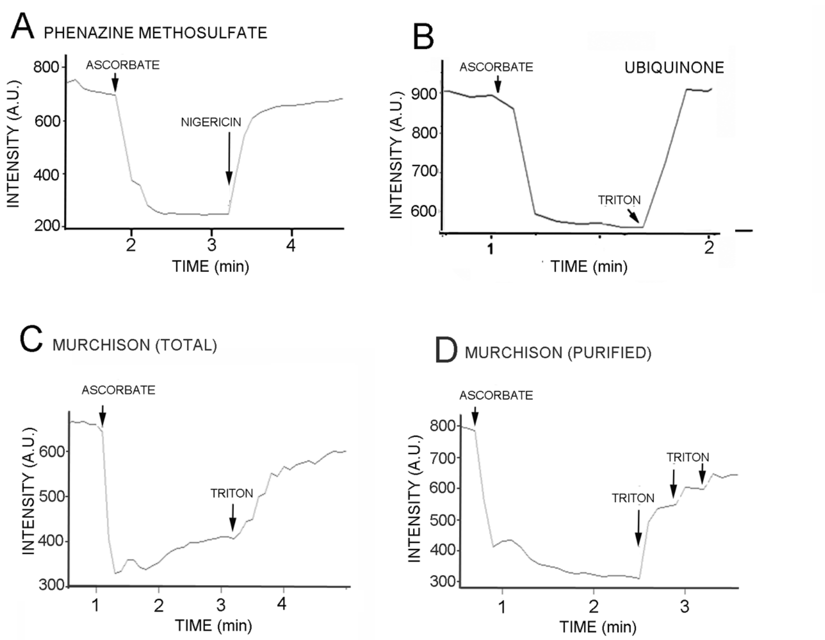
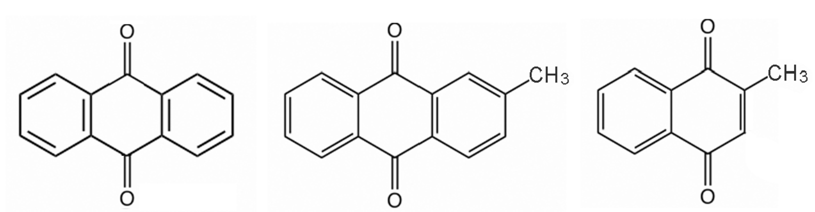


Figure 3. Measuring pH gradients with 9-aminoacridine fluorescence quenching. If a pH gradient exists across a liposome membrane, acidic inside, 9-aminoacridine accumulates in the internal volume and its fluorescence becomes quenched. The amount of quenching is related to the magnitude of the gradient and the total internal volume which allows ΔpH to be calculated [9].

The results convinced us that one or more compounds were present that could transport electrons and protons across lipid bilayer membranes, but what could they be? To answer this question, we initiated a collaboration with George Cooper at the NASA Ames Research Center. Cooper uses GC-MS (gas chromatography-mass spectrometry) to analyze organic compounds in carbonaceous meteorites. He soon discovered that three quinones were present: 9,10-anthraquinone, 2-methyl-9,10-anthraquinone and 2-methyl-1,4-naphthoquinone (Figure 4). These quinones, either alone or as a mixture, would be capable of generating proton gradients across liposome membranes that separate a reducing agent outside and an oxidant inside.



9,10-anthraquinone 2-methyl-9,10-anthraquinone 2-methyl-1,4-naphthoquinone

Figure 4. Quinones present in the Murchison meteorite.

6. Conclusions

Ever since the Murchison and other carbonaceous meteorites were discovered to contain multiple organic compounds relevant to life, including amino acids, nucleobases and membrane-forming amphiphiles, their composition has been used as a guide to the kinds of organic compounds that would have been available to support the origin of life. The guidance is then used to develop hypotheses about how the compounds could combine and react on the early Earth and ultimately emerge as living systems. However, it must be said that origins of life research is like putting together a jigsaw puzzle. The frame is established by the laws of chemistry and physics, but just a few pieces so far have been fitted together inside the frame so the "big picture" is not yet visible. We have added one more piece to the puzzle by showing that quinones are present in carbonaceous meteorites and demonstrating how they could have used the energy of a redox potential to generate chemiosmotic proton gradients in the earliest forms of life. Just as life today takes up essential amino acids and vitamins from the environment, early life could have incorporated quinones from the environment as nutrients that were not required to be synthesized metabolically as they are in life today.

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