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BRIEF COMMUNICATION

Acidity of the thylakoid lumen in plastids makes sense from an evolutionary perspective

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Abstract

An acid pH in the lumen of chloroplast thylakoids is necessary in order to derive the required amount of CO_2 to account for the observed rates of carbon fixation. We point out that the endosymbiotic derivation of the chloroplast from a cyanobacterium would have resulted in the lumen of the thylakoid having an acid pH. The thylakoids of cyanobacteria are continuous with the plasma membrane, resulting in the lumen of the thylakoid being open to the outside of the cell. Endosymbiosis resulted in the cyanobacterium being taken up into a food vacuole of a protozoan. The vacuole would have had an acid pH, probably around pH 5, so the endosymbiotic bacterium would have been surrounded by an environment with an acidic pH. The lumen of the thylakoids would have been at an acid pH since they were open to the exterior of the cell, and to the contents of the vacuole.

Additional key words: carbon fixation; carbon concentrating mechanism; ATPase.

Carbon occurs in water as dissolved inorganic carbon (DIC) which is composed of HCO_3^-, CO_3^{2-}, and CO_2 (Kasting and Walker 1991):

$$H_2O + CO_2 \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^- \leftrightarrow 2\ H^+ + CO_3^-$$

Alkaline conditions drive the reactions to the right, while acidic conditions drive the reactions to the left. The chloroplast stroma has a pH ranging from 7.4 to 7.8 (Raven 1997). At this pH, almost all of the inorganic carbon is present as HCO_3^- (see Fig. 5.1 in Falkowski and Raven 1997). The amount of CO_2 in the chloroplast stroma under these conditions is too small to account for the rates of observed carbon fixation by ribulose bisphosphate carboxylase/oxygenase (RuBPCO). RuBPCO occurs in the chloroplast stroma and will only fix inorganic carbon as CO_2. In order to account for the observed rates of carbon fixation, it has been proposed that the

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lumen of thylakoids is maintained at a pH of 5.0 (Pronina *et al.* 1981, Pronina and Semenenko 1990, 1992, Pronina and Borodin 1993, Raven 1997). In this model, dissolved inorganic carbon diffuses through the cytoplasm, into the chloroplast stroma, and through the thylakoid membrane as HCO_3^-. In the thylakoid lumen, HCO_3^- is converted into CO_2, either non-enzymatically or by carbonic anhydrase. At this pH, CO_2 is about 20 times more abundant than HCO_3^- (see Fig. 5.1 in Falkowski and Raven 1997). CO_2 then diffuses from the lumen of the thylakoid, through the thylakoid membrane into the chloroplast stroma, the site of RuBPCO, where carbon is fixed. The main strength of the above hypothesis is its ability to account for the observed rate of carbon fixation.

There is a great deal of evidence to support the acidity of the lumen of the thylakoids in light. Photosynthetic water oxidation by photosystem 2 results in the formation of one O_2 and four H^+ from two H_2O molecules (Haumann and Junge 1994). This reaction is catalyzed by a tetranuclear manganese cluster located at the luminal side of the thylakoid membrane (Förster and Junge 1985, Haumann and Junge 1994). The proton gradient across the thylakoid membrane is used to drive ATP synthesis according to the chemiosmotic theory of Mitchell (1966).

In this paper, we show that the acidity of the lumen of the thylakoid is logical if one examines the evolutionary origin of the chloroplast. It is widely accepted that the chloroplast evolved by an endosymbiotic event involving a cyanobacterium (blue-green alga) and a phagocytic protozoan (Mereschkowsky 1905). In this event, the cyanobacterium was taken up into the food vacuole of the phagocytic protozoan (Fig. 1). Instead of being digested, however, the cyanobacterium was maintained as an endosymbiont in the vacuole, providing the host with a portion of its photosynthate. Subsequent evolution resulted in the endosymbiotic cyanobacterium evolving into a chloroplast.

A closer examination of the sequence of events leading to the evolution of the chloroplast shows how acidity of the thylakoid lumen could have occurred. Thylakoids in cyanobacteria are continuous with the plasma membrane (Pankratz and Bowen 1963, Jost 1965, Fuhs 1966, Smith and Peat 1967, Allen 1968a,b). Indeed one cyanobacterium, *Gloeobacter violaceus*, does not have thylakoids and, instead, has phycobilisomes and presumably the rest of the radiant energy-capturing apparatus on the plasma membrane (Rippka *et al.* 1974). These observations have led to the conclusion that thylakoids in cyanobacteria evolved by invagination of the plasma membrane.

In the endosymbiotic theory of evolution of the chloroplast, a cyanobacterium was taken up into the food vacuole of a phagocytic protozoan, where the cyanobacterium became endosymbiotic and eventually evolved into a chloroplast. Vacuoles are kept acidic with a pH of about 5.0 by vacuolar proton-translating ATPases (V-ATPases) in the vacuolar membrane (Mellman *et al.* 1986, Klionsky *et al.* 1990, Nakamura *et al.* 1997, Tomashek *et al.* 1997). It is, therefore, probable that the endosymbiotic cyanobacterium resided in an acidic environment in the vacuole of the phagocytic protozoan.

Since the thylakoids of the endosymbiotic cyanobacterium were continuous with the plasma membrane, the lumen of the thylakoids was open to the acidic contents of the protozoan vacuole, in which the endosymbiotic cyanobacterium resided (presuming the cell wall of the cyanobacterium, which was lost in the evolution to the chloroplast, was not a barrier to passage of the contents of the protozoan vacuole —Fig. 1). Thus, in this endosymbiosis, the lumen of the thylakoids of the cyanobacterium would have been acidic. In addition, the vacuole of the host protozoan would have contained a number of different enzymes, such as carbonic anhydrase, with acidic pH optima.

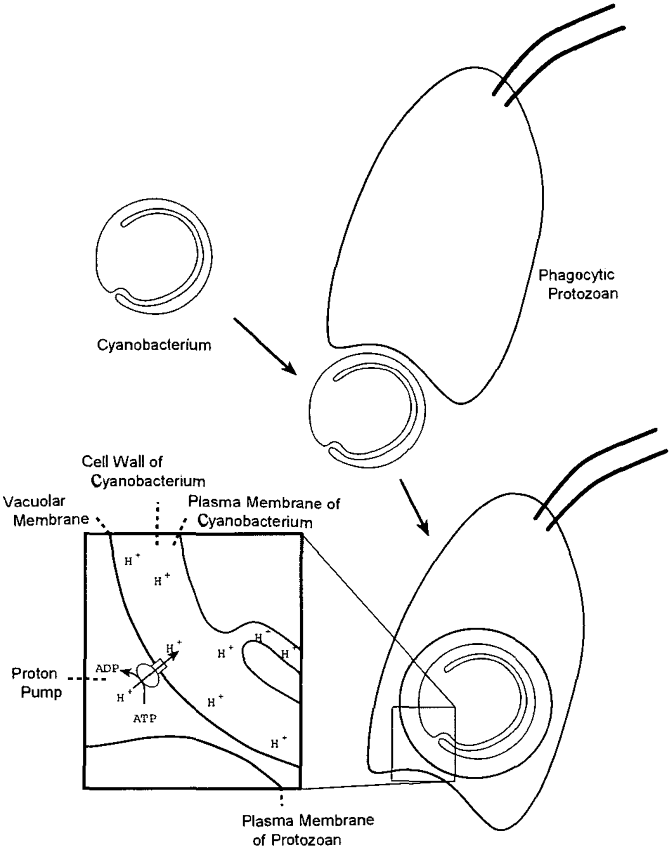

Fig. 1. The evolutionary sequence leading to acidity of the thylakoid lumen in an endosymbiotic cyanobacterium. A free-living cyanobacterium was taken up into the food vacuole of a phagocytic protozoan. Instead of being digested, however, the cyanobacterium became an endosymbiont living in the acidic environment of the vacuole. The thylakoids were continuous with the plasma membrane of the cyanobacterium, exposing the lumen of the thylakoids to the acidic contents of the vacuole, thereby resulting in acidity of the lumen of the thylakoids.

Subsequent evolution resulted in the endosymbiotic cyanobacterium evolving into a chloroplast. The thylakoids of the endosymbiotic cyanobacterium with their acidic lumens would have evolved into the thylakoids of the chloroplast. The thylakoids of developing extant chloroplasts arose from invaginations of the inner membrane of the chloroplast envelope, probably reflecting the origin of chloroplasts from an endosymbiotic cyanbacterium.

The evolutionary origin of thylakoid activity infers that there are three mechanisms for maintaining an acid pH between membranes in chloroplasts (Fig. 2):

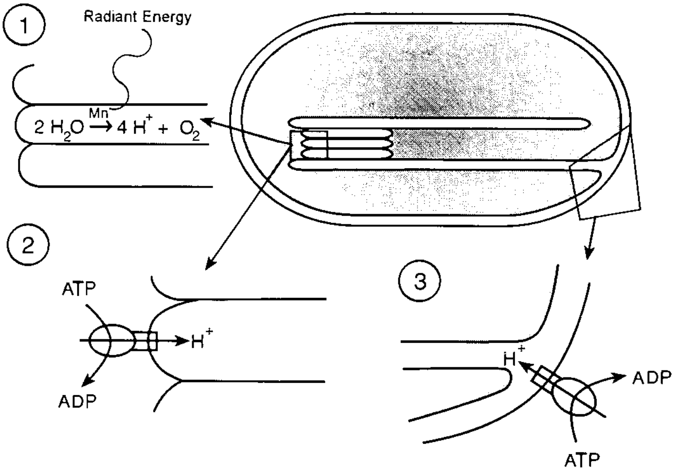

Fig. 2. Three possible mechanisms that could operate to maintain acidity between membrane spaces in chloroplasts. *1*. Light photooxidizes water in the lumen of the thylakoid. *2*. F-ATPases in the thylakoid membrane pump H^+ into the lumen of the thylakoid at the expense of ATP. *3*. V-ATPases in the outer membrane of the chloroplast envelope pump H^+ into the space between the inner and outer membrane of the chloroplast envelope at the expense of ATP.

(*1*) The first mechanism operates only in the light when two molecules of water are photooxidized to four protons and O_2.

(*2*) The second mechanism utilizes F-ATPases (F_0-F_1 type ATPases) in the thylakoid membrane to pump protons into the lumen of the thylakoid at the expense of ATP (Nelson and Taiz 1989).

(*3*) The third mechanism ultilizes V-ATPases (Nelson and Taiz 1989) embedded in the outer membrane of the chloroplast envelope to pump protons at the expense of ATP into the space between the inner and outer membrane of the chloroplast envelope. Although not shown experimentally, this third mechanism would make sense based on the endosymbiotic origin of chloroplasts discussed earlier. The food vacuole membrane of the phagocytic protozoan would maintain the vacuolar contents at an acid pH by means of V-ATPases. The food vacuole membrane of the phagocytic protozoan evolved into the outer membrane of the chloroplast envelope with the proton-pumping V-ATPases in the membrane.

The presence of V-ATPases in the outer membrane of the chloroplast envelope would have two important implications in regard to photosynthesis:

(*1*) The space between the inner and outer membrane of the chloroplast envelope would be maintained at an acid pH by the expenditure of cytoplasmic ATP by the V-ATPases in the outer membrane of the chloroplast envelope. The use of cytoplasmic ATP, instead of chloroplast ATP, would make it possible to maintain the acidity of the chloroplast membrane spaces in the dark, since the cytoplasmic ATP could come from glycolysis or Krebs cycle reactions.

(*2*) The presence of an acidic space between the inner and outer membrane of the chloroplast envelope would stabilize the dissolved inorganic carbon mostly in the form of CO_2. This is important since CO_2 is the only form of dissolved inorganic carbon that can be used by RuBPCO. Diffusion of CO_2 into the stroma of the chloroplast, location of RuBPCO, would result in the fixation of CO_2.

A protozoan with an endosymbiotic cyanobacterium would have had a selective advantage in an environment where CO_2 was limiting, *e.g.*, such as the marine environment of today, where at a pH of 8.2, a minimal amount of inorganic carbon is in the form of CO_2. Seawater in equilibrium with the atmosphere at pH 8.2 and 25 °C has about 2 000 μM of dissolved inorganic carbon. At this pH, 10 μM occurs as CO_2, 200 μM occurs as CO_3^{2-} with the remainder occurring as HCO_3^- (Falkowski and Raven 1997). In such an environment, HCO_3^- would diffuse from the surrounding seawater, through the cytoplasm and chloroplast stroma, and into the lumen of the thylakoids. Here the acidic pH would result in conversion of the inorganic carbon to principally CO_2, which would diffuse through the thylakoid membrane to RuBPCO in the chloroplast stroma.

The evolution of a photosynthetic eukaryote in the Precambrian, with an endosymbiotic cyanobacterium/chloroplast, would have resulted in an organism that would have been able to outcompete the existing cyanobacteria in environments low in dissolved CO_2, *e.g.*, the open oceans of the time. The photosynthetic eukaryote, by virtue of thylakoids with acidic lumens, would be able to convert HCO_3^- in the seawater to CO_2, and thereby fix carbon at a faster rate than free-living cyanobacteria. In such a scenario, the green and red algae (the direct descendants of the protozoan with an endosymbiotic cyanobacterium) would have come to dominate the ancient oceans of the Precambrian.

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