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 CO₂ 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 HCO₃⁻, CO₃²⁻, and CO₂ (Kasting and Walker 1991):

\[ \text{H₂O} + \text{CO₂} \leftrightarrow \text{H₂CO₃} \leftrightarrow \text{H}^+ + \text{HCO₃}^- \leftrightarrow 2 \text{H}^+ + \text{CO₃}^- \]

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 HCO₃⁻ (see Fig. 5.1 in Falkowski and Raven 1997). The amount of CO₂ 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 CO₂. 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$_2$O 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 photosynthesize. 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