Małgorzata Rydzy

Title of PhD thesis: “Structural and functional characteristic of chaperone proteins involved in RuBisCo biosynthesis”

Abstract

Ribulose-1,5-bisphosphate carboxylase / oxygenase (Rubisco) is the most abundant protein in the biosphere. This enzyme is responsible for attaching atmospheric CO_2 to ribulose-1,5-bis-phosphate (RuBP) during the Calvin Benson Bassham cycle, also known as the dark phase of photosynthesis. The subsequent reaction of this cycle results in the formation of two 3-phosphoglyceric acid molecules (3PGA). This compound is the starter for many anabolic pathways. In addition to the carboxylation reaction, Rubisco can also carry out the oxygenation reaction (O_2 attachment to RuBP). As a result of this reaction, phosphoglycolate is formed, which is toxic to the cell. It is therefore an unfavorable reaction in which the cell loses energy as ATP. Besides the oxygenation reaction, another limiting factor of the photosynthesis is the fact that Rubisco is a slow enzyme, meaning it can only perform a few carboxylation reactions per second. It is also the only enzyme in nature that enables the incorporation of inorganic into organic matter on a large scale.

Due to the fact that Rubisco is a slow and inaccurate enzyme, methods to improve its kinetic parameters have been sought for years. A major obstacle to this endeavor is Rubisco's complex biosynthesis, which makes it difficult and sometimes impossible to test potentially kinetically better enzyme mutations. Rubisco comes in four different forms, of which form I, found in algae, cyanobacteria and higher plants, is the most abundant on Earth.

Until recently, it was impossible to obtain active plant Rubisco in bacterial cells. in 2017, a group of scientists was the first to present the heterologous plant expression of Rubisco in *E.coli* cells, which required the simultaneous co-expression of five chaperones. Interestingly, cyanobacterial Rubisco, which is also form I (similar to the plant version), is expressed in bacteria with only one cyanobacterial chaperone RbcX (or without in some cases). However, Rubisco derived from *Synechocystis* sp. PCC6803 is not functionally expressed in bacteria, suggesting that an additional factor is required for this process.

It is believed that proteins from the Hsp40 / 70 family, i.e. DnaJ and DnaK proteins, may be involved in the Rubisco biosynthesis. So far, there has been only one reference to this topic in the literature, saying that the expression of cyanobacterial Rubisco in bacteria involves the DnaJ / DnaK / GrpE proteins from *E.coli*. However, no specialized cyanobacterial homologs of these proteins have been identified. In S*ynechocystis* sp. PCC6803, seven homologs of the DnaJ protein and three DnaK proteins are encoded.

The aim of this study was: to identify whether proteins from the Hsp40 and Hsp70 families are involved in the Rubisco biosynthesis process from *Synechocystis* sp. PCC6803.