

RUBISCO FROM THERMOPHILIC CYANOBACTERIA
Thermosynechococcus elongatus

RAFAL BARTOSZEWSKI¹, GÜNTER F. WILDNER², JAROSŁAW
KRÓLICZEWSKI¹ and ANDRZEJ SZCZEPANIAK¹

¹Department of Biophysics, Institute of Biochemistry and Molecular Biology
University of Wrocław, Poland, ²Plant Biochemistry, Ruhr-University Bochum,
D-44780 Bochum, Germany

The importance of ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco, EC 4.1.1.39) would be difficult to exaggerate, because it provides the only quantitatively significant link between the pools of inorganic and organic carbon in biosphere. The major reason for paying so much attention to rubisco stems from the fact that it catalyzes the rate-limiting step in photosynthesis. In 1971 it was discovered that this protein catalyses an additional reaction involving molecular oxygen, oxygenation of ribulose-1,5-bisphosphate. It is believed to catalyze the first reaction in the process of photorespiration which essentially wastes assimilated carbon. In photorespiration the enzyme combines with oxygen, rather than carbon dioxide, to create a compound that is subsequently converted into carbon dioxide. In other words, rubisco catalyzes one reaction that incorporates carbon into plants and other that ultimately strips them of carbon. Under current atmospheric conditions, potential photosynthesis in C₃ is suppressed by oxygen by as much as 40%. The potential for increasing net CO₂ fixation, by increasing carboxylase or decreasing oxygenase, makes rubisco an obvious target for genetic engineering, but we have failed to make rubisco better, despite more than 20 years of effort. We simply do not yet know enough about the rubisco structure-function relationships or about those of proteins in general, to design a better enzyme *de novo*. Genetic selection for better enzyme is also not feasible because more than few amino acids need to be change. However that is reason for optimism. Hope comes from the discovery that some diatoms and red algae have more-specific (better) rubisco than in higher plants. Moreover the rubisco the termophilic red algae rubisco was found to be about three times more efficient. Perhaps we could swap parts of good rubisco with part of poor enzyme, or find better rubisco in some species and transfer them to crop species that have poorer rubisco.

Diatoms and red algae have a red-like type of rubisco while higher plants and cyanobacteria have a green-like type of rubisco. Swaping of part green-like rubisco with part red-like seams to be very difficult. Also the transfer of red-like rubisco to species having green-like type of rubisco is difficult and the first approach was unsuccessful. Therefore, the aim of our proposal is looking for better green-like type rubisco. Our preliminary results suggest that the termophilic cyanobacteria (*Thermosynechococcus elongatus* and *Phormidium laminosum*) have an interesting enzyme. To fulfill this project the following objectives are proposed: Objective 1) Overexpression in *E. coli* of recombinant

ribulose-1,5-bisphosphate carboxylase/oxygenase from *Thermosynechococcus elongatus*; 2) Determination of kinetic value of rubisco.

So far rubisco operon from *Thermosynechococcus elongatus* has been cloned into pUC18 plasmid, and then expressed in *Escherichia coli*. Also procedures for isolation the enzyme from this expression system and for rubisco further purification up to above 80 percent of electrophoresis homogeneity, has been developed and some kinetic parameters (K_{mRUBP}) established.