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Re-engineering of RuBisCO for the purpose of producing algal biofuels may be a lost cause

Svetoslav Dimitrov Alexandrov

Proponents of algal biofuels hope that photosynthesis could be improved, which would make them economically feasible. One of the means to do that would be by engineering and making the slow enzyme RuBisCO more efficient. In this commentary article I discuss the possibility it could be a lost cause, because the proper place of the enzyme in the evolutionary history is still misunderstood.

The interest in algal biofuels was only moderate prior to 2006-2007 [1], after which the publications of research articles on this subject bloomed. One of the articles that facilitated the ignition of the scientific debate was written by Chisti in 2007 [2] and as of 29 August 2016, this article has been cited totally 6276 times, including self-citations (Google Scholar Data). Regardless of the hype, the prospects of biofuel production from microalgal biomass are unrealistic in the near future. Research teams in our department have published two articles on the subject– in 2012 [3], concerning biodiesel, and in 2015 [4], concerning other types of algal fuels: bioethanol, biohydrogen, biogas, hydrocarbons, and direct burning of algal biomass. In both manuscripts the conclusions are clear: mass production of algal biomass and its subsequent treatment to extract valuable components remain difficult and expensive and as such, algae should be best utilized as food and not as fuel.

Now I'd like to add more to the discussion of algal biofuels by focusing on only one of the significant obstacles. If we would really like to produce biofuels in a fast and efficient way, a primary goal would be to fixate CO_2 as quickly into organic compounds as possible. This leads straight to the point. The enzyme RuBisCO, responsible for the first major step of the carbon fixation, is very slow, being only able to fix 3 CO_2 molecules per second. Not only that - the problem with RuBisCO is also tied to its oxygenase ability. Petkov *et al* [3] have already pointed out that if the algae are not supplied with additional CO_2, after the depletion of the naturally available CO_2 the enzyme starts using O_2 as a substrate due to its oxygenase ability. As a result photorespiration levels are higher, and the quantity of the biomass is low. Regrettably, supplying algae with additional CO_2 adds to the cost. Chatterjee & Basu [5] discuss the possibilities of reengineering and improving RuBisCO, as they describe it as "the world's most abundant protein is perhaps the most incompetent enzyme". The authors claim that familiar tools of the Green Revolution are providing diminishing returns and according to them RuBisCO is a tempting target.

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There is certain research dedicated to re-engineer the RuBisCO and changing CO2/O2 specificity factor. Unfortunately, results from experiments with cyanobacteria are so far met with mixed success. Durall and Lindbald [6] have written a very detailed review article about engineering approaches in cyanobacteria for increased carbon fixation. Concerning RuBisCO substitutions of certain amino acids may lead to an increase of the specificity factor, but simultaneously to a decrease of CO_2 fixation rate. Some experiments lead to a decrease of the specificity factor and there are also some who report both increase of the specificity factor and the CO_2 fixation rate. But overall, these researchers say that "it has not been possible to significantly improve the RuBisCO specificity for CO_2, the affinity to CO_2 and/or the carbon fixation rate", "the enzyme may already be optimized through evolution", and "it still remains unclear if a significant improvement of the carboxylation by RuBisCO can be achieved". Could the enzyme be indeed already optimized? This is a very interesting question that needs to be discussed in-depth. Evolutionary, RuBisCO is described as a relic of a bygone age, it has evolved under conditions different from these of today and it hasn't changed significantly since then [7]. This could easily make some researchers think that RuBisCO is probably maladapted and non-efficient in its function. But could it be that it is simply misunderstood? This question was asked by Morell *et al* [8] in 1992, more than two decades ago, but since scientists continue suggesting RuBisCO could be improved, probably it's still relevant today. Researchers may hope that engineering methods would significantly improve RuBisCO's performance, however, if it hasn't already happened before by natural means, is this hope justified? Here it must be reminded that during the later stages of the Earth history, internal CO_2 concentration mechanisms appeared, for example, the C4 mechanism in higher plants [9]. It seems that evolutionary forces pushed neither for drastic modifications of the structure of RuBisCO, nor for an alternative catalytic system. Instead, they pushed for supplementary mechanisms that concentrate CO_2 and saturate RuBisCO's catalytic capacity.

Conclusion

I suggest the engineering efforts should focus on improving these, rather than touching RuBisCO. But so far the goal to create fast growing algae which are independent of additionally supplied CO_2 remains unmet and I still think that the future of obtaining biofuels from algal biomass remains bleak.

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