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DEVELOPMENT OF THE SYSTEM FOR HETEROLOGOUS SYNTHESIS AND MATURATION OF HYDROGENASES, THE MOST EFFICIENT BIOHYDROGEN PRODUCERS

K. Sybirna

Laboratory of Fundamental Mechanisms in Bioenergetics
Institute of Biology and Technology of Saclay
Unit of Bioenergetics, Structural Biology and Mechanisms
CEA Saclay, 91191 Gif-sur-Yvette, France
e-mail: k_sybirna@yahoo.com

Biohydrogen is considered as an important key to a sustainable power supply and is currently seen as a versatile fuel of the future, with the potential to replace fossil fuels. The most efficient biohydrogen producers are metaloenzymes hydrogenases that reversibly catalyse the reaction: $H_2 \leftrightarrow 2H^+ + 2e^-$. Due to inefficient homologous expression, the envisaged strategy for hydrogenase production is the use of a heterologous system, which, however, is hindered by the complex maturation processes of these enzymes. The limit of our understanding in this field makes a barrier for hydrogenases' application in a variety of biotechnological processes (Sybirna K, Bottin H., 2013). This work focuses on the construction of a new efficient system for heterologous expression and maturation of the [Fe-Fe] hydrogenase from eukaryotic green alga *Chlamydomonas reinhardtii* (Sybirna K, Antoine T., Lindberg P., et al., 2008) with further enhancement of its catalytic activity (Sybirna K., Ezanno P., Baffert C., et al., 2013).

Based on codon usage bias and hydrogenase maturation ability, the bacterium *Shewanella oneidensis*, which possesses a putative [Fe-Fe] hydrogenase operon, was used as a host for *C. reinhardtii* [Fe-Fe] hydrogenase production (Sybirna K, Antoine T., Lindberg P., et al., 2008). The purified algal hydrogenase was fully mature and homogeneous in that it bore the predicted full complement of iron. Thus, among the existing systems for heterologous [Fe-Fe] hydrogenase expression and maturation, the one based on *S. oneidensis* is the most efficient.

For *C. reinhardtii* [Fe-Fe] hydrogenase HydA1 the question of catalytic activity and electron transport is of main importance. It is known that this enzyme directly accepts electrons from ferredoxin which is mediated by some surface residues on hydrogenase. A mutant form of *C. reinhardtii* hydrogenase was found where aspartic acid in place of arginine 171 leads to a six-fold increase of the catalytic activity in comparison to the wild type protein. At the same time this mutation leads to a strong decrease in ferredoxin-dependent hydrogen production while the catalytic center of mutant forms stays intact (Sybirna K., Ezanno P., Baffert C., et al., 2013). This study highlights the role of electrostatic interactions, via arginine 171, between HydA1 and ferredoxin, the effect of its mutation on electron transfer and, most importantly, the fact that electron transfer to hydrogenase catalytic center is a limiting step in H_2 production.

The understanding that in the wild-type enzyme H_2 production is strongly limited by the electron transfer step will help to create novel enzymes with improved catalytic activity for biotechnological purposes.

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