Towards photosynthesis-driven H_2 production using O_2-tolerant [NiFe]-hydrogenases

Oliver Lenz
Institute of Biology / Microbiology, Humboldt-Universität zu Berlin, Chausseestrasse 117, 1015 Berlin, Germany, oliver.lenz@cms.hu-berlin.de

Photoystem I releases electrons at a sufficiently low redox potential (ca. -450 mV) that allows the direct reduction of protons to molecular hydrogen (H_2). The latter process is catalyzed by the metalloenzyme hydrogenase. However, most hydrogenases are extremely sensitive to even traces of O_2. This feature makes them unusable for the direct connection to the oxygenic photosynthesis.

Fortunately, some microbes are able to energize their aerobic respiration chain with electrons derived from the oxidation of molecular hydrogen. This facultative process is mediated by the so-called "oxygen-tolerant" [NiFe]-hydrogenases, which are heterodimeric proteins containing multiple cofactors that require a sophisticated maturation machinery.

By genetic engineering, we have coupled *in vitro* the O_2-tolerant membrane-bound [NiFe]-hydrogenase from *Ralstonia eutropha* to photosystem I from *Synechocystis* PCC6803 in a way that allows efficient electron transfer between both components. In fact, upon illumination and by using a sacrificial electron donor, the PS I –hydrogenase fusion produced H_2 rate that significantly exceeded those of comparable systems.

These results represent a significant step for implementation of a functional PS I-hydrogenase hybrid complex in a cyanobacterium that is able to produce H_2 directly from the light-dependent process of water oxidation.

Ludwig, M., Cracknell, J. A., Vincent, K. A., Armstrong, F. A., Lenz, O. 2009. Oxygen-tolerant H_2 oxidation by membrane-bound [NiFe] hydrogenases of *Ralstonia* species. Coping with low level H_2 in air. *J. Biol. Chem.* 284: 465-477.

Cracknell, J.A., Wait, A. F., Lenz, O., Friedrich, B., Armstrong, F.A. 2009. A kinetic and thermodynamic understanding of O_2 tolerance in [NiFe]-hydrogenases. *Proc. Natl. Acad. Sci. U S A.*

Lenz, O., Ludwig, M., Schubert, T., Bürstel, I., Ganskow, S., Goris, T., Schwarze, A., Friedrich, B. 2010. H_2 conversion in the presence of O_2 as performed by the membrane-bound [NiFe]-hydrogenase of *Ralstonia eutropha*. *Chem. Phys. Chem.* 11: 1107-1119.

Schwarze, A., Kopczak, M.J., Rögner, M. Lenz, O. 2010. Requirements for construction of a functional hybrid complex of photosystem I and [NiFe]-hydrogenase. *Appl. Environ. Microbiol.* 76: 2641-2651.

Krassen, H., Schwarze, A., Friedrich, B., Ataka, K., Lenz, O., Heberle, J. 2009. Photosynthetic hydrogen production by a hybrid complex of photosystem I and [NiFe]-hydrogenase. *ACS Nano.* 22: 4055-4061.