

What green algae are up to in the dark: Researchers decipher little-known metabolic pathway for hydrogen production

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How green algae produce hydrogen in the dark is reported by biologists at the Ruhr-Universität Bochum in the "*Journal of Biological Chemistry*". Hereby, they have uncovered a mechanism for the production of the gas which has hardly been examined before; usually, researchers are interested in light-driven hydrogen synthesis. "Hydrogen could help us out of the energy crisis", says Prof. Dr. Thomas Happe, head of the working group Photobiotechnology. "If you want to make green algae produce more hydrogen, it is important to understand all the production pathways."

Green algae produce hydrogen under stress - even in the dark

Single-celled [green algae](#) of the type *Chlamydomonas* are microscopically small organisms: ten of them fit side by side on a human hair. In some ways, microalgae are not so very different from higher plants, such as trees. For example, they also perform [photosynthesis](#). Unlike [land plants](#), they can use light energy for the production of [molecular hydrogen](#) (H₂). "However, *Chlamydomonas* and co only form [hydrogen](#) under stress", says Thomas Happe. "The disposal of the energy-rich gas serves as a kind of overflow valve so that excess [light energy](#) does not damage the sensitive photosynthetic apparatus." *Chlamydomonas* can also produce hydrogen in the dark. Although this fact has been known for decades, H₂ synthesis in the absence of light

has barely been studied because much less of the gas is produced in the dark than in the light. Moreover, it is complicated to isolate large quantities of the key enzyme of the dark-reaction, the so-called pyruvate:ferredoxin oxidoreductase. The RUB researchers nevertheless tackled the project.

Hydrogen production in the dark mimicked in vitro

Happe's team reconstructed the core of the dark [hydrogen production](#) in vitro, thus demonstrating the underlying mechanism. In order to get to the proteins involved, the researchers had these produced by bacteria. First they introduced the corresponding genes of the green algae into the gut bacterium *Escherichia coli*, for example, the gene for the pyruvate:ferredoxin oxidoreductase. *E. coli* then produced the proteins according to this blueprint. Happe's team isolated them from the bacterial cells and examined them like a construction kit. In the test tube, the biologists analysed how different combinations of proteins interacted with each other under specific environmental conditions.

"Ancient enzyme" discovered

In so doing, they found out that, under stress in the dark, the algae switch to a [metabolic pathway](#) which is normally only found in bacteria or single-celled parasites. "Chlamydomonas has an evolutionarily ancient enzyme", explains Jens Noth from the working group Photobiotechnology. "With the help of vitamin B1 and iron atoms, it gains energy from the breakdown of sugars." This energy is then used by other green algal enzymes, the hydrogenases, to form hydrogen. The unicellular microalgae switch on this metabolic pathway when they suddenly encounter oxygen-free conditions in the dark. Because, like humans, the green algae need oxygen to breathe if they cannot draw their energy from sunlight. The formation of hydrogen in the dark helps the

cells to survive these stress condition. "With this knowledge, we have now found another piece of the puzzle to get an accurate picture of H₂ production in *Chlamydomonas*", says Thomas Happe. "In future, this could also help to increase the biotechnologically relevant light-dependent H₂ formation rate."

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