

Green hydrogen production using algal proteins

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Bioreactors with green algae could be ecologically cleanest source of hydrogen.
 Pictured above: Dr. Adam Kubas from the Institute of Physical Chemistry of the
 Polish Academy of Sciences in Warsaw, one of the co-authors of the publication

in *Nature Chemistry*. Credit: IPC PAS, Grzegorz Krzyzewski

We are increasingly thinking about hydrogen as a successor of crude oil—for instance, through the use of hydrogen fuel cells. But where will the hydrogen come from? Industrial or domestic bioreactors using green algae could be the ecologically cleanest source. An international team of researchers has, for the first time, precisely described the chemical reactions responsible for the stability of hydrogen generation in an aerobic environment by algal enzymes.

Water can be photosynthetically split by algae operating in suitable conditions. However, there is a serious obstacle standing in the way of efficient, green bioreactors: Hydrogenases, the enzymes that are directly responsible for the production of hydrogen, are inactivated in the presence of oxygen. Would it be possible to make them resistant to the effect of the atmosphere? Until now, this was not possible, because the mechanism of their degradation by oxygen was not sufficiently well understood. Now, these complex processes have been fully described for the first time and published in the journal *Nature Chemistry* by a team of scientists from Poland, France, Great Britain, Spain and the United States.

"Hydrogen is an element that is widespread in our environment. But its universality does not mean that it is readily available in large quantities, using ecologically clean methods," notes Dr. Adam Kubas from the Institute of Physical Chemistry of the Polish Academy of Sciences (IPC PAS) in Warsaw, one of the co-authors of the publication. He gives the following example: "The simplest method of hydrogen production seems to be electrolysis of water, but it is only simple and fairly ecological if clean power is available. Meanwhile, the power generated currently comes into being in processes that have little in common with real

ecology."

The road to ecologically pure hydrogen resources leads through water—and more specifically, by what floats in it. Algae are a comprehensive group of microorganisms that process water and carbon dioxide by the usual process of photosynthesis, and as a result, emit oxygen into the environment. It arises due to enzymes known as hydrogenases. The reactions occur in a complex active site. A key role is played by two atoms of iron (Fe-Fe) or an atom of nickel and an atom of iron (Ni-Fe).

Since the late 1990s, it has been known that when there is no sulfur in the algae's environment, it produces hydrogen instead of oxygen. Researchers quickly realized that in natural conditions, algal enzymes are one of the best hydrogen catalysts. Unfortunately, hydrogenases have a serious drawback: When exposed to oxygen they undergo degeneration and stop working. Meanwhile, providing them with an anaerobic environment in industrial or domestic conditions would be very difficult.

The authors of the publication in *Nature Chemistry* investigated reactions occurring in the iron-iron (Fe-Fe) hydrogenases. The researchers were particularly interested in identifying the migration paths of the [oxygen molecules](#) from the surroundings to the active site within the protein structure and understanding the course of the reactions they were involved in there.

"Our study rather resembled searching for the way through an unknown maze, with the difference that ordinary mazes are large and effectively two-dimensional, whereas ours was three-dimensional and microscopically small. In addition, we could not look directly inside it! Experimental data only told us the speed of some of the electrochemical reactions occurring in hydrogenases under specified conditions," explains Dr. Kubas.

Simulations of gas transport were conducted at University College London (UCL), the University of Cambridge, and CIC nanoGUNE in San Sebastián. Quantum chemical calculations associated with the transformations of oxygen in the active Fe-Fe site were conducted by Dr. Kubas at UCL, and completed at the IPC PAS. The theoretical results were confronted with the electrochemical experiments on hydrogenases, conducted mostly at the Centre National de la Recherche Scientifique in Marseille.

The research team proposed and verified the full catalytic cycle associated with the reactions of oxygen in the active site of the hydrogenases. Two pathways along which oxygen molecules penetrated into the site were identified in the structure of the proteins, wherein one of them proved to be frequented particularly often. The availability of electrons in the protective mechanism was also found to play a key role.

"Why have electrons become important? It turns out that when they are absent in the active site—reactions with oxygen lead to the formation of hydroxyl radicals, which damage the protein. However, if electrons are available, oxygen can be reduced to completely harmless water," explains Dr. Kubas.

In order to confirm the soundness of the new catalytic model, the researchers proposed making some small modifications to the structure of the hydrogenases. The substitution of one of the small amino acids with a bulky one on the path of transport of [oxygen](#) was expected to hinder the migration of gas molecules to the [active site](#). Careful experiments with appropriately mutated hydrogenase confirmed these assumptions and validated the model.

Research on hydrogenases at the IPC PAS is now being continued under the SONATA grant from the Polish National Science Centre. In the coming years, its aim will be to determine the natural regulation

mechanisms of electron transport to prevent the creation of destructive [hydroxyl radicals](#). If the work is successful, the first high-performance and fully ecological bioreactors producing hydrogen can be expected in about a decade.

More information: Adam Kubas et al, Mechanism of O₂ diffusion and reduction in FeFe hydrogenases, *Nature Chemistry* (2016). [DOI: 10.1038/nchem.2592](#)

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