Downloaded from rsta.royalsocietypublishing.org

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A
MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Photolysis of Water for H<latex>$_{2}$</latex> Production with the Use of Biological and Artificial Catalysts

D. O. Hall, M. W. W. Adams, P. Morris and K. K. Rao

Phil. Trans. R. Soc. Lond. A 1980 **295**, 473-476
doi: 10.1098/rsta.1980.0143

Email alerting service Receive free email alerts when new articles cite this article - sign up in the box at the top right-hand corner of the article or click **here**

To subscribe to *Phil. Trans. R. Soc. Lond. A* go to: **http://rsta.royalsocietypublishing.org/subscriptions**

This journal is © 1980 The Royal Society

Downloaded from rsta.royalsocietypublishing.org

Phil. Trans. R. Soc. Lond. A **295**, 473–476 (1980)
Printed in Great Britain

Photolysis of water for H_2 production with the use of biological and artificial catalysts

BY D. O. HALL, M. W. W. ADAMS, P. MORRIS AND K. K. RAO
Department of Plant Sciences, King's College, University of London, London SE24 9JF, U.K.

An aqueous mixture of chloroplasts, hydrogenase and an electron transfer catalyst on illumination liberates H_2, the source of the H atoms being water. The rate and duration of H_2 production from such a system depends on the stability of chloroplast and hydrogenase activities in light and oxygen. Both chloroplasts and hydrogenases can be stabilized to a certain degree by immobilization in gels or by incubation in bovine serum albumin. Natural electron carriers of hydrogenases are ferredoxin, cytochrome c_3 and NAD. Viologen dyes and synthetic iron–sulphur particles (Jeevanu) can substitute for the biological carriers. Methyl viologen, photoreduced in the presence of chloroplasts, can liberate H_2 in combination with Pt (Adam's catalyst). An aqueous solution of proflavine can be photoreduced in the presence of organic electron donors such as EDTA, cysteine, dithiothreitol, etc.; the reduced proflavine can subsequently liberate H_2 with MV-Pt, MV-hydrogenase, ferredoxin-hydrogenase or cytochrome-hydrogenase systems.

Photolysis of water for producing hydrogen fuel has three advantages not shown by other energy systems:

(*a*) the supply of the substrate, water, is unlimited,
(*b*) the source of energy, Sun, is free and perennial, and
(*c*) the product, hydrogen, is a storable and non-polluting source of energy.

Photolytic hydrogen production can be brought about by the use of organic photosensitizers and metal or enzyme catalysts or by a modification of the process of photosynthesis. Our work is based mainly on the latter process, namely biophotolysis of water. An aqueous suspension of chloroplast membranes isolated from plants or algae on illumination splits water into oxygen and reducing equivalents. Krampitz (1972) reported that this reducing power generated from illuminated spinach chloroplasts can be coupled with methyl viologen and crude *Escherichia coli* hydrogenase to liberate molecular hydrogen. A year later Benemann *et al.* (1973) demonstrated that spinach chloroplasts mixed with ferredoxin and *Clostridium kluyveri* hydrogenase evolved hydrogen in the light. The hydrogen evolution occurred without any added electron donors suggesting that the source of H atoms may be water. In the past four years we have been investigating the characteristics of this *in vitro* system and have shown by the use of suitable controls that water is the electron donor for H_2 production. We have also increased considerably the yield of hydrogen from such an *in vitro* system (Rao *et al.* 1975, 1978*a*, *b*).

The photosynthetic electron flow from water to hydrogenase resulting in the liberation of molecular hydrogen is shown in figure 1. The rate and extent of hydrogen production from such a system will depend upon (*a*) the ability of chloroplast membranes to function continuously in light, (*b*) the capacity of hydrogenase to function continuously in the presence of

The Royal Society is collaborating with JSTOR to digitize, preserve, and extend access to
Philosophical Transactions of the Royal Society of London. Series A, Mathematical and Physical Sciences.
www.jstor.org

Downloaded from rsta.royalsocietypublishing.org

oxygen and (*c*) the ability of the ferredoxin to shuttle electrons between the photosystems of chloroplasts and the hydrogenase.

Isolated chloroplasts are sensitive to light, heat and oxygen. At ambient temperatures and normal daylight their photosynthetic electron transport activity decays at a very fast rate; this decay of chloroplast function is further enhanced by the presence of oxygen. The stability of chloroplasts in light varies to a certain extent on the species from which they are extracted – out of a number of plants tested the chloroplasts of *Chenopodium quinoa* were found to have the best stability. Fixation of chloroplast membranes using glutaraldehyde or using protein cross-linking agents did not improve their capacity to function continuously in light. However, addition of 0.5–1 % bovine serum albumin to isolated chloroplasts did improve their functional half life. The oxygen liberated at Photosystem II, if not removed from the reaction mixture, can oxidize components of the chloroplast membranes resulting in a loss of chloroplast function. Indeed, addition of oxygen and peroxide scavengers (glucose, glucose oxidase, catalase and ethanol mixture) increased the longevity of the hydrogen evolution system (Hall *et al.* 1978).

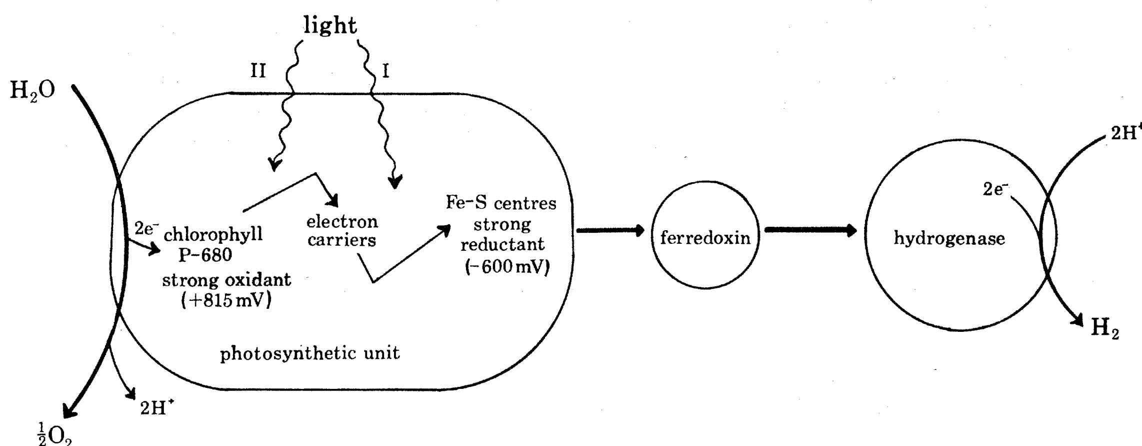

FIGURE 1. Coupling of solar energy to H_2 production by using stabilized chloroplast membranes and hydrogenase enzymes.

Hydrogenases have been isolated in recent years from a variety of bacteria. The enzyme from *Clostridium pasteurianum* is very active and so far is the best hydrogenase known to give maximum yields of hydrogen from a chloroplast–ferredoxin system. However, the enzyme is extremely oxygen sensitive. There are many hydrogenases stable in oxygen, but they do not readily couple with reduced ferredoxin. This is not a serious drawback as these hydrogenases have been found to react readily with other reduced electron carriers such as cytochrome c_3, NADH or methyl viologen (Rao *et al.* 1978*b*). Thus, by using a system containing isolated *Chenopodium* chloroplasts, bovine serum albumin, oxygen and peroxide scavengers, bacterial hydrogenases and suitable electron mediators in the optimal concentrations, we can now produce hydrogen from our system at a rate of 40–50 μmol/h per mg chlorophyll.

Biological materials, when isolated from their natural environment, are intrinsically unstable at ambient conditions and it is desirable to replace them with synthetic, stable substitutes if possible. With this object in mind we have been trying to replace chloroplasts, ferredoxin or hydrogenase in our system by other catalysts. Synthetic tetranuclear analogues of the active sites (Fe_4S_4) of ferredoxins, which have been chemically reduced with sodium dithionite, will

Downloaded from rsta.royalsocietypublishing.org

couple to hydrogenase and evolve hydrogen (Adams *et al.* 1977). However, these analogues were unable to replace hydrogenase in the above chloroplast system.

The ability of platinum to reversibly activate molecular hydrogen has been known for many years (Green & Stickland 1934; Krasna 1961). In a system containing chloroplasts and methyl viologen the electron carrier, platinum dioxide (Adam's catalyst), would replace hydrogenase and catalyse hydrogen production. Control experiments showed platinum was a true catalyst producing hydrogen at a rate of about 10 μmol/h per mg chlorophyll. Similar rates were obtained with purified *E. coli* hydrogenase (Adams & Hall 1978). The limiting factor in H_2 production was that methyl viologen at high concentrations (> 50 μM) caused inhibition.

Table 1. Platinum catalysed hydrogen evolution from a photoreduction system

electron donor	H_2 produced after 20 h/μmol	percentage yield
EDTA (120 mM)	6.3	5.2
dithiothreitol(10 mM)	5.5	55
dithiothreitol (50 mM)	12.0	24
glutathione (10 mM)	2.5	25
glutathione (100 mM)	6.7	6.7
cysteine (10 mM)	3.5	35
mercaptoethanol (0.7 M)	8.8	1.3

The 2 ml reaction mixture contained proflavine (40 μM), methyl viologen (1 mM) and platinum (PtO_2, 100 μg) and the electron donor in phosphate–citrate buffer, pH 6.0, in 15 ml sealed vials under O_2-free N_2. The vials were incubated at 30 °C and illuminated (light intensity, 11000 lux) to start the reaction.

Table 2. Platinum and hydrogenase catalysed hydrogen evolution from photoreduced electron carriers

catalyst	electron carrier	H_2 evolved in 24 h/μmol
C. pasteurianum hydrogenase	*S. maxima* ferredoxin (25 μM)	7.8
C. pasteurianum hydrogenase	*C. pasteurianum* ferredoxin (3 μM)	9.3
D. gigas hydrogenase	*D. desulfuricans* cytochrome c_3 (3 nM)	10.8
C. pasteurianum hydrogenase	Jeevanu C	0.21
C. pasteurianum hydrogenase	Jeevanu D	0.18
platinum	Jeevanu C	0.12
platinum	Jeevanu D	0.13

The 2 ml reaction mixture contained proflavine (40 μM), EDTA (120 mM) and the catalyst and electron carrier as indicated. The amounts used were: PtO_2, 100 μg; *C. pasteurianum* hydrogenase, 24 units; *D. gigas* hydrogenase, 36 units; Jeevanu C and D, 250 μg. The reaction was carried out as in table 1. Jeevanu materials were a gift from Dr Ranganayaki and were prepared by exposing to light a mixture of mineral salt solutions containing Fe, Mn, Mo and various sulphur anions, amino acids and formaldehyde (Bahadur 1975).

Methyl viologen can be photoreduced by irradiation with visible light in the presence of the photosensitizer proflavine and EDTA as the electron donor (Sweetser 1967). Under continuous illumination, platinum catalysed the evolution of substantial quantities of hydrogen from this system for up to 24 h. EDTA could be replaced by cysteine, glutathione, mercaptoethanol or dithiothreitol (table 1). With 120 mM EDTA plus 50 mM glutathione as electron sources, the platinum-catalysed photoproduction of hydrogen continued over 50 h, with the production of 54 μmol of H_2 (32% yield). The turnovers of methyl viologen and proflavine were 54 and 1350 respectively. The photodestruction of the proflavine was the limiting factor of the longevity

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

Downloaded from rsta.royalsocietypublishing.org

of H_2 production as on addition of fresh proflavine to the latter system after 50 h, hydrogen evolution continued for a further 50 h. In the absence of proflavine, methyl viologen or platinum there was no hydrogen production. We have thus shown that these catalysts will evolve hydrogen over prolonged periods; the replacement and recycling of the electron donors by a water-splitting reaction is an important goal of the future.

Ferredoxin and cytochrome c_3 were also photoreduced in the presence of proflavine and EDTA. Illumination in the presence of *C. pasteurianum* hydrogenase or *Desulphovibrio gigas* hydrogenase (for which cytochrome c_3 is the natural electron carrier) led to the evolution of hydrogen at very high rates (table 2). Platinum would not replace the hydrogenases in these systems. Hydrogenase and platinum would also evolve hydrogen from the photochemically produced electron carriers (Jeevanu) on irradiation in the presence of proflavine and EDTA, but the quantities of H_2 evolved were very low (table 2).

In conclusion, by selecting the right catalysts at their optimum conditions it is possible to capture solar radiation and transfer its energy into hydrogen gas. The problem of chloroplast instability has to be tackled before the biophotolytic system can be applied for any large scale production of hydrogen from water. An understanding of the biological systems seems essential (since it is the only one that functions in visible light at present) if we are to attain our goal of a completely synthetic system for the evolution of H_2 gas from water.

This work was supported by grants from the Commission of the European Communities, I.C.I. Ltd and the Science Research Council.

References (Hall *et al.*)

Adams, M. W. W., Reeves, S. G., Hall, D. O., Christou, G., Ridge, B. & Rydon, H. N. 1977 *Biochem biophys. Res. Commun.* **79**, 1184–1191.

Adams, M. W. W. & Hall, D. O. 1979 *Biochem. Soc. Trans.* **6**, 1339-1361.

Bahadur, K. 1975 *Zobl. Bakt. Abt* II, Bd **130**, S211–218.

Benemann, J. R., Berenson, J. A., Kaplan, N. O. & Kamen, M. D. 1973 *Proc. natn. Acad. Sci. U.S.A.* **70**, 2319–2320.

Green, D. E. & Stickland, L. H. 1934 *Biochem. J.* **28**, 898–900.

Hall, D. O., Rao, K. K. Reeves, S. G. & Gogotov, I. N. 1978 In *Alternative Energy Sources* (ed. T. N. Veziroglu), pp. 3675–3696. Washington, D.C.: Hemisphere Publishing.

Krampitz, L. O. 1973 In *Biophotolysis of water*. N.S.F.-RANN report HA_2N 73-014 quoted by Benemann in *Living systems as energy converters* (ed. R. Buve *et al.*), p. 288. Amsterdam: North Holland.

Krasna, A. J. 1961 *J. Am. chem. Soc.* **83**, 289–291.

Rao, K. K., Gogotov, I. N. & Hall, D. O. 1978*a* *Biochimie* **60**, 291–296.

Rao, K. K., Morris, P. & Hall, D. O. 1978*b* In *Hydrogenases, their catalytic activity, structure and function* (ed. H. G. Schlegel), pp. 439–452. Göttingen: E. Goltze.

Rao, K. K., Rosa, L. & Hall, D. O. 1976 *Biochem. biophys. Res. Commun.* **68**, 21–27.

Sweetser, P. B. 1967 *Analyt. Chem.* **39**, 979–982.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY A MATHEMATICAL, PHYSICAL & ENGINEERING SCIENCES