# PROBLEMS IN THE STABILIZATION OF THE IN VITRO PHOTOCHEMICAL ACTIVITY OF CHLOROPLASTS USED FOR $H_2$ PRODUCTION

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## Introduction

The combination of biocatalysts for photolysis of water with an auxiliary enzyme system to generate $H_2$ and/or reduced organic compounds has lent interest to the extent to which stabilization of components of such a system could be achieved [1]. Biological $H_2$-generating systems at several levels of organization can be envisaged (fig.1) by joining together (a) individual enzymes and catalysts; (b) catalysts in organized units, as membranes, cell fragments, or cells; (c) different cells as the coupling of algae with photosynthetic bacteria, where the excreted carbon of algae is the substrate for $H_2$ production by photosynthetic bacteria, or an in vivo system wherein these requirements are present within a single organism, e.g., filamentous blue-green algae containing spatially separated cells specialized for photosynthesis and nitrogen fixation where nitrogenase can function as a hydrogenase; or (d) artificial catalysts patterned after the natural systems.

## Results and discussion

$H_2$ production by chloroplasts–hydrogenase system is shown in table 1. Similar to the previous reports [2,3] the system requires light, chloroplasts supplement

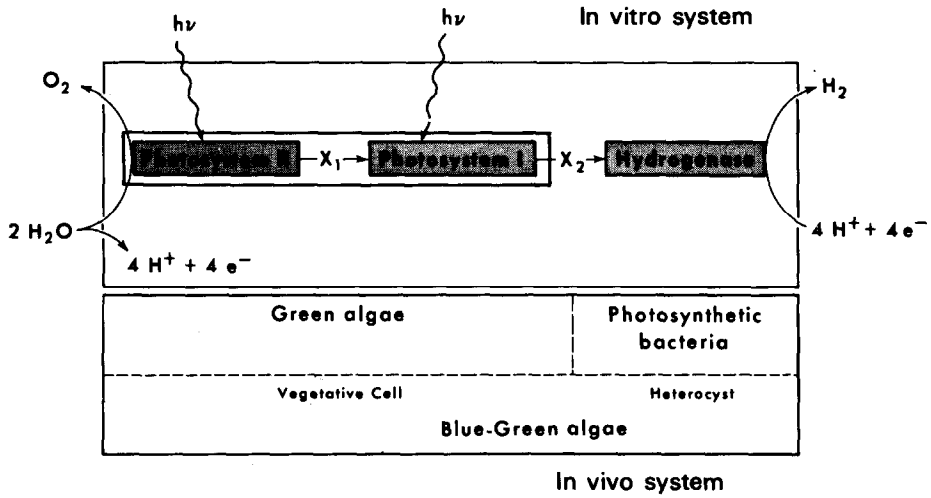


Fig.1. Scheme for biological solar energy conversion using in vitro and in vivo systems for $H_2$ generation. In vitro systems, solid lines; in vivo systems, dashed lines. $X_1$ designates electron carriers linking the photosystems together. For the in vitro system, $X_2$ designates a low potential electron acceptor produced by the system which has a potential sufficient to couple to the hydrogenase system. For the in vivo system, $X_2$, designates reduced carbon compounds excreted by algae. These serve as hydrogen donors to photosynthetic bacteria which convert $X_2$ into a low potential substance that drives their hydrogenase.

Table 1
Light dependent $H_2$ production in a chloroplast–hydrogenase coupled system using water as the electron donor

| Changes in basic system | $H_2$ evolution μmoles/mg Chl/h |
|---|---|
| None | 2.8 |
| – Hydrogenase | 0 |
| Dark | 0 |
| + Catalase + ethanol | 8.1 |
| With 20 μl hydrogenase | 5.3 |
| + 5 mM ascorbate | 2.7 |
| – Ferredoxin | 0 |

Basic system: spinach chloroplasts isolated in 0.33 M sorbitol medium (400 μg chlorophyll), 20 nmol spinach ferredoxin, 50 mmol glucose, 10 units glucose oxidase, 50 μl hydrogenase (550 μmol $H_2$/ml/h in dithionite assay) and 0.1 M HEPES buffer, pH 7.5, volume of 2 ml at 25°C. Illumination 5000 ft candles. Na-ascorbate (5 mM), catalase (1000 units) and ethanol (10 μl) as indicated. $H_2$ rates calculated from 1-h readings.

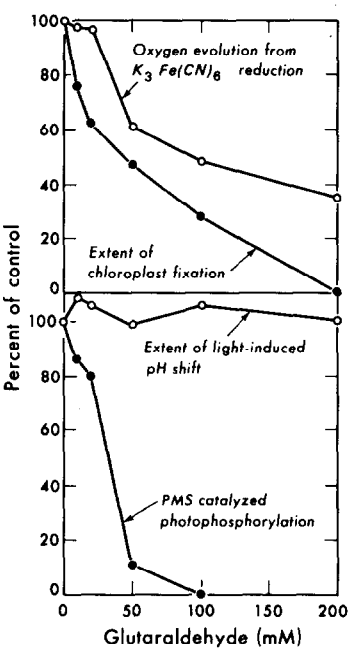


Fig.2. Effect of glutaraldehyde fixation of pea chloroplasts upon photosynthetic electron transport with water as the electron donor and upon proton gradients (after West and Packer, [12]).

ed with ferredoxin and hydrogenase and an $O_2$ trapping system. In other studies we have shown its dependence upon both photosystem II and I. Also bovine serum albumin addition enhances activity and stability at 15°C where continuous light-dependent $H_2$ evolution for 24 h can be obtained with initial rates greater than 40 μmol $H_2$/mg Chl/h (I. Fry, S. Sarma, K. Rao, collaboration, [4]). Some of the problems in stabilizing this system are the relative instability of the photosystems and hydrogenase to oxygen and light [1,5–7]. This requires research to protect the native system or to develop stable analogs patterned after these catalysts. Various ways of solving these problems are currently being considered such as to arrange the components such that $O_2$ and $H_2$ are evolved spatially separated.

One approach to stabilization which has had some success in enzyme technology is to immobilize enzymes to surfaces or gels, resulting in differing degrees of success in extending the functioning lifetime of enzymes. Treating chloroplasts with two types of bifunctional cross-linking reagents show some promise. Chloroplasts cross-linked with glutaraldehyde [8–13] lose their osmotic responses, but retain unexpectedly high photo-induced electron transport across photosystems II and I, and transmembrane proton gradients [12], but such immobilized chloroplasts cannot synthesize ATP or incorporate $CO_2$ into carbohydrates. As shown in fig.2, isolated pea chloroplasts treated with glutaraldehyde progressively decrease their osmotic response, but electron transfer and proton gradients are largely unaffected [9].

Recently, (G. Papageorgiou collaboration, [14,15]) we have also studied bifunctional imidoesters, another type of crosslinking agent. Imidoesters show selectivity for reactivity with primary $NH_2$ groups, and amidination introduces a positively charged imino group for each $NH_2$ reacted, leaving electrical charge largely unaltered. Photosystem II activity is lost by dimethylsuberimidate treatment at 20–25°C, but photosystem I activity is resistant and is even slightly stimulated from 1350 ± 150 to 1700 ± 180 μequiv/mg Chl/h. However, photosystem II activity is not lost if the treatment is made at 0–4°C for 14 to 18 h. At 40–50 μmol/mg Chl, the osmotic response is frozen to a lower but constant value, while electron transport reaches a maximum of 170–200 for treated chloroplasts and 50–70 μequiv/mg Chl/h for untreated controls which are comparable to the rates of freshly prepared

chloroplasts. When dimethylsuberimidate treated chloroplasts are subjected to intermittent illumination, ferricyanide-supported $O_2$ evolution is detectable even after 12–15 repetitions of the light–dark cycles, i.e., for a total of 16–20 min exposure to light, but rates are 1/3 to 1/5 less in controls which lose photosystem II activity more readily.

Light-induced proton gradients are also retained after cross-linking with imidoesters as with bifunctional aldehydes. Our laboratory has also demonstrated [16] that light-induced proton gradients can be retained for more than 40 days in cold-stored spinach chloroplasts supplemented with scavengers for free radicals (butylated hydroxytoluene) and free fatty acids (bovine serum albumin). Furthermore, chloroplasts stored at 15°C on prolonged storage lose their light-dependent ability to develop proton gradients readily, but after cross-linking with dimethylsuberimidate or glutaraldehyde retain this activity for longer periods [17].

## Summary

Illuminated chloroplasts can be coupled to hydrogenase to produce $H_2$ using water as the electron donor but the system is unstable. Chloroplasts cross-linked with bifunctional reagents as dimethylsuberimidate and glutaraldehyde can withstand somewhat better than untreated controls the loss upon storage of photosystem II and I activity and light-induced proton gradients. Further studies are required on the stabilization of these catalysts in vitro and in vivo before a long term system for the production of $H_2$ under continuous illumination can be developed.

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