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The photobiocatalytic system: inorganic semiconductors coupled to bacterial cells

A.A. Krasnovsky and V.V. Nikandrov

A.N. Bach Institute of Biochemistry, Academy of Sciences of the USSR, Leninsky prospect, 33, 117071 Moscow, USSR

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Considering the evolutionary pathways of solar energy conversion, we suggested that some mineral components of the earth's crust might have been capable of electron phototransfer coupled to the metabolic chains of primary heterotrophic cells. We observed a peculiar type of this process when light-exposed particles of titanium dioxide or some other minerals initiated an electron transfer from organic electron donors to *Clostridium butyricum* bacterial cells, with hydrogen being evolved as a result of intra- and extracellular activity of hydrogenase. The coupling of mineral photocatalysts to bacterial cells may be regarded as a way to create a new type of photobiocatalytic system.

Hydrogen evolution; Titanium dioxide; Solar energy conversion; (*Clostridium* cell)

1. INTRODUCTION

Efficient charge separation in the reaction centers is the primary step of solar energy conversion in photosynthesis. To construct model systems, photoreceptors capable of charge separation may be employed, provided they are coupled to the electron transfer chain of cell structures or isolated enzymes (review [1]).

For primary charge photoseparation, the particles of inorganic photocatalysts-semiconductors were used. The electron and the hole formed under the action of light were localized in the active centers on the phase boundary of the photocatalyst particles suspended in the water medium.

In 1962 our laboratory proposed an inorganic model of the Hill reaction [2]. We used an aqueous suspension of photocatalysts (TiO_2, ZnO, W_2O_3), ferric compounds and *p*-benzoquinone as electron acceptors, and water molecules as primary electron donors. The quantum yield of molecular oxygen photoproduction here reached 3%, and the stoichiometry corresponded to that of the Hill reaction in chloroplasts. The success of these experiments was determined by the fact that the semiconductor particles could act both as a light energy-absorbing photoreceptor and as a catalyst on the surface of which the initially formed active products were stabilized and transformed.

Later, we observed photoreduction of MV^{2+} in a TiO_2 suspension, exogenous electron donors enhanced the efficiency of the process [3].

Formation of the 'blue' radical of reduced methyl viologen (MV^+) was a precondition for molecular hydrogen photoreduction, if bacterial hydrogenase preparations were introduced into the suspension. Such experiments involved the TiO_2 suspension [4] as well as CdS particles [5].

It was recently shown in our laboratory [6,7] that in the TiO_2-electron donor system, the coupling of the semiconductor to hydrogenase is needed for hydrogen evolution if the 'blue' methyl viologen radical is formed, whereas in the case of two-electron MV^{2+} photoreduction no exogenous hydrogenase is needed for hydrogen production.

Correspondence address: A.A. Krasnovsky, A.N. Bach Institute of Biochemistry, Academy of Sciences of the USSR, Leninsky prospekt, 33, 117071 Moscow, USSR

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Here a system is described in which the particles of inorganic catalysts-semiconductors are coupled to hydrogenase-containing *Clostridium butyricum* bacterial cells. To facilitate the electron transfer between the mineral photocatalyst particles and the bacterial cells, MV^{2+} capable, in the reduced state, of lipid membrane penetration was used as a relay.

2. MATERIALS AND METHODS

Cells of *C. butyricum* were cultivated in a glucose-peptone medium containing 10 g/l glucose, 10 g/l peptone, 2 g/l K_2HPO_4, 2 mg/l $FeSO_4 \cdot 5H_2O$ and 0.01 mg/l biotin. Spores were introduced into the sterile medium and kept for 12 h at 37°C. The cultivated cells were separated from the medium by centrifugation at 1600 × g, transferred into a 0.5 M sucrose solution and incubated under gaseous argon at 3°C, to keep them intact. The absence of hydrogenase activity measured by hydrogen evolution from the reduced MV^{2+} in the supernatant after cell centrifugation showed that the cells were intact. In some cases the cells were washed with a 0.5% glucose solution and subsequently centrifuged, then, diluted with the same solution, the cell suspension was pipetted into a Dewar vessel filled with liquid nitrogen, where the frozen drops were stored. Before application, the frozen drops were thawed in a stream of argon, and this led to cell destruction. The rate of hydrogen photoproduction was high enough to use a manometric Warburg apparatus, supplied with glass vessels and a quartz mercury lamp PRK-4 placed on the bottom of the vessel. The lamp was in a glass tube and immersed into the thermostatic bath of the Warburg apparatus (Hg lines at 365 nm were mainly efficient, intensity 10^5 erg/cm^2 per s).

Usually, a sample of the photocatalyst (50–300 mg), 0.1 ml of 10^{-3} M aqueous solution of MV^{2+}, 0.3 ml of a suspension of intact or disrupted cells (3 mg protein) and no more than 6 ml of a 0.05–1.0 M solution of the organic electron donor were placed into a Warburg vessel. A 40% solution of CO_2-absorbing KOH was placed into the side arm of the vessel. Experiments were carried out at 25°C. TiO_2 (anatase), CdS and ZnO were of chemical grade.

3. RESULTS AND DISCUSSION

The most efficient H_2 photoproduction was observed in the system: Tris-TiO_2-MV^{2+}-bacterial cells. The rate of hydrogen evolution in the system reached 5–15 μl/min, depending on the hydrogenase activity of the cells. According to the preliminary measurements, the quantum yield of hydrogen photoproduction reached 10%. Hydrogen evolution in the light proceeded at a constant rate for several hours until the electron donor was exhausted, for the hydrogenase activity of the clostridial cells was stable under UV illumination and anaerobic conditions.

Experiments on a gas chromatograph confirmed that the evolved gas was hydrogen. In the absence of Tris, TiO_2 or bacterial cells, H_2 evolution did not exceed 0.1 μl/min. When the inactivated clostridial cells were used, no hydrogen production was observed (cell inactivation had been achieved by heating at 100°C or exposing to UV light in the air). Hydrogen photoproduction registered in the Tris-TiO_2 system was less than 0.5 μl/min, and this is in good agreement with our previous measurements [6,7].

When the intact cells were used, the exclusion of MV^{2+} from the complete Tris-containing system led to a 2–2.5-fold decrease in the rate of H_2 evolution. If ruptured clostridial cells were used, the introduction of MV^{2+} exerted little, if any, effect on H_2 evolution in the TiO_2 suspension in Tris buffer. In the course of photochemical experiments a partial destruction of the clostridial cells took place, as indicated by the appearance of some hydrogenase activity in the supernatant obtained by centrifugation of the TiO_2 suspensions containing bacterial cells after 30 min pre-illumination.

Thus, in the course of the experiment, two hydrogenase forms must have been functioning: one located inside the cells, and the other leaking from the cells as a result of cell destruction. The possibility of direct contact of hydrogenase with semiconductor particles was suggested in our previous observations [2,6,7], and in the recent experiments where hydrogenase binding to TiO_2 was shown [8]. In systems containing intact clostridial cells, their coupling to photocatalyst particles may proceed via reduced methyl viologen capable of cell membrane penetration (fig.1). However, excretion of the low-molecular mass electron carrier

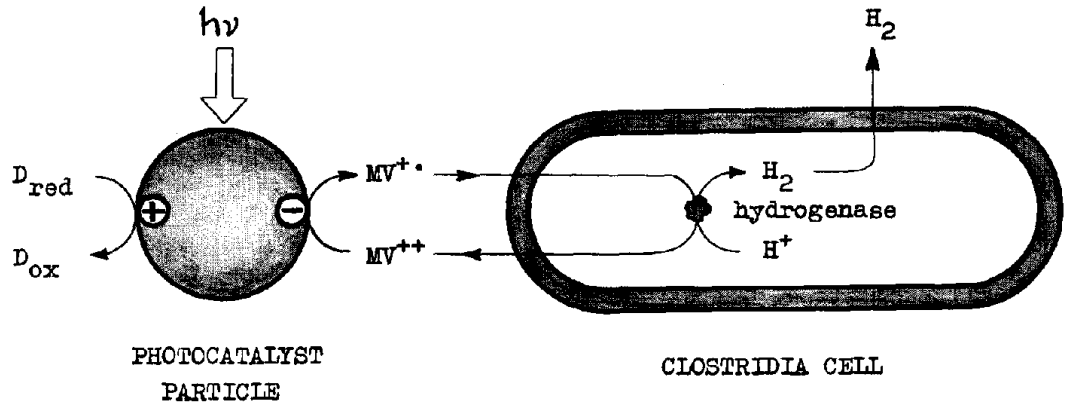

Fig.1. Scheme for hydrogen photoproduction in the coupling of mineral photocatalyst to clostridial cells. MV^{2+}, methyl viologen; D_{red} and D_{ox}, reduced and oxidized electron donor, respectively.

by the bacterial cells cannot be excluded either. But this is the subject of a special study. With all the electron donors listed, except Tris buffer, H_2 photoproduction had low efficiency in the absence of MV^{2+}. If glucose was present in the reaction medium, hydrogen evolution from clostridial cells was activated under UV illumination [9], but the rate of this process was insignificant compared with the TiO_2-containing systems. ZnO and CdS coupled to the clostridial cells appeared to be less efficient sensitizers of H_2 photoevolution than TiO_2. In suspensions of CdS, hydrogen evolution was observed only with glycine and ethanol as electron donors. It should be noted that in contrast to ZnO and CdS, TiO_2 formed stable suspensions in Tris-buffer solutions.

Table 1

Hydrogen photoproduction in the system: mineral photocatalyst–*Clostridium butyricum* cells

Electron donor	TiO_2	ZnO
Tris (0.05 M, pH 7.2)	18.4	1.8
Tricine (0.05 M, pH 7.0)	6.1	–
Glycine (0.2 M, pH 5.5)	7.0	–
Glucose (1 M)	16.1	2.3
Carboxymethylcellulose (60 mg)	1.4	–
Glycerol (1 M)	15.6	2.0
Ethanol (10%)	4.0	3.1
Methanol (10%)	6.0	4.0
Without electron donor	0.0	0.0

Reaction medium (6 ml) contained 50 mg TiO_2 or 300 mg ZnO, 5×10^{-4} M MV^{2+}, *C. butyricum* cells (3 mg protein), and an organic electron donor. Experiment lasted for 60 min. Illumination with 365 nm lines of mercury lamp, light intensity 10^5 erg·cm^{-2}. Rate of H_2 photoevolution given in μl H_2/3 min

The above experiments may be helpful in constructing artificial photobiological systems and may also serve as a model of possible evolutionary pathways of solar energy conversion. The fact that mineral compounds of the earth's crust could have been used as replicating matrixes [10] and primary photocatalysts in the course of chemical evolution has been reported [11]. According to Oparin's hypothesis [12], the primary organisms could be heterotrophs of the clostridial type, and gradual evolution towards the use of solar energy could have required photoreceptors. The above experiments illustrate such a peculiar possibility, perhaps, a blind alley of evolution.

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