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Efficient photoinduced electron transfer from inorganic semiconductor TiO_2 to bacterial hydrogenase

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Hydrogenase from *Thiocapsa roseopersicina* adsorbed on TiO_2 particles, using electrons photogenerated in the conduction band of the semiconductor, in the presence of an organic electron donor, carries out hydrogen production with the rate comparable to that obtained with reduced methyl viologen as the substrate. Under conditions of the efficient hydrogenase adsorption on TiO_2 (1.6 mM $CaCl_2$, 0.05 M Tris buffer, pH 7.2) the quantum efficiency of hydrogen photoproduction is up to 10%. Coupling of inorganic semiconductors with bacterial hydrogenase may be regarded as a way to the development of artificial photocatalytic systems of light energy conversion.

Hydrogen photoproduction; Titanium dioxide; Hydrogenase; Solar energy conversion

1. INTRODUCTION

The coupling of the inorganic semiconductors capable to generate, under irradiation, electron-donor and electron-acceptor centres at the phase boundary with the enzymes providing reactions which involve photogenerated charges, is a possible approach to the development of photocatalytic systems of solar energy conversion.

Previously, in our laboratory hydrogen photoevolution was revealed in aqueous suspensions of TiO_2, ZnO, CdS containing electron donor, bacterial hydrogenase and methyl viologen [1–4]. Also the possibility of TiO_2 coupling with *Clostridia* cells containing hydrogenase was found [5,6]. Cuendet et al. studied H_2 photoproduction in similar systems with bacterial hydrogenases immobilized on TiO_2 particles [7,8]. Recently, NAD(P) photoreduction and photosynthesis of organic and amino acids in semiconductor suspensions containing enzymes were reported [9]. In the systems investigated the electron transfer from semiconductor to the enzyme was provided by electron mediators: methyl viologen (MV^{2+}), *Rhodium bipyridyl* complexes, etc.

In suspensions of TiO_2 and ZnO a low efficient H_2 photoproduction was observed in the absence of electron mediator [1,3–6], which indicated a direct electron transfer from the semiconductor to the active centre of the enzyme. The possibility of H_2 photoproduction in the absence of electron mediator in TiO_2 suspensions containing electron donors and bacterial hydrogenases was also confirmed by other authors [8]. It remains, however, unclear whether an effective energy conversion can be achieved in the case of the mediator-less coupling of the enzyme with the semiconductor.

In the present paper the conditions of the direct photoinduced electron transfer from TiO_2 particles to hydrogenase from *Thiocapsa roseopersicina* which provide hydrogen photoproduction with quantum efficiency up to 10% were established.

2. MATERIALS AND METHODS

In the experiments dispersed TiO_2 powder (anatase) (8.5 m^2/g) from Fluka and P25 TiO_2 (50 m^2/g) from Degussa were employed. The suspensions of semiconductors were prepared in 0.05 M buffer solution of Tris(hydroxymethyl)aminomethane (Tris), pH 7.2, and in solutions of sucrose, dithiothreitol and 0.05 M phosphate buffer, pH 7.2.

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Double distilled water was used. Hydrogenase from phototrophic bacteria *T. roseopersicina*, strain BBC, was isolated and purified according to the procedure described [10]. Hydrogenase activity when assayed at 20°C was 2.4 μl $H_2 \cdot min^{-1} \cdot ml^{-1}$ in the reaction mixture containing: hydrogenase, 0.6 mg protein per ml; MV^{2+}, 2 mM; potassium dithionite, 30 mM; 0.05 M Tris buffer, pH 7.2.

To measure the amount of the evolving H_2, 1 ml of semiconductor suspension containing 33 mg TiO_2 or 12 mg P25 TiO_2 and 0.6 mg hydrogenase was placed in a glass vial fitted with crimped rubber septa. The suspensions were deoxygenated by flushing with argon, and then irradiated with a Hg lamp in the region 320–800 nm (2.8×10^5 erg $\cdot cm^{-2} \cdot s^{-1}$) or Hg lines 365 nm ($1 \times 10^4$ erg $\cdot cm^{-2} \cdot s^{-1}$) under stirring. Hydrogen evolved was assayed by gas chromatography using molecular sieve 5 Å column. Light intensity was measured with a ferrioxalate actinometer. Calculating the quantum yield of H_2 photoproduction, we assumed the incident light (365 nm) to be completely absorbed by the semiconductor suspension.

3. RESULTS AND DISCUSSION

Under irradiation of TiO_2 suspension containing hydrogenase and Tris buffer as electron donor, H_2 was produced (table 1). This is in good agreement with the data of our previous studies [1]. However, the reaction rate under these conditions was by two orders lower than that in the presence of MV^{2+} as electron mediator. This, apparently, was accounted for by the low efficiency of the direct transfer of the photogenerated electrons from semiconducting particles to the enzyme molecules.

We studied the influence of the chlorides of mono- and bivalent metals on H_2 photoproduction in the absence of electron mediator. It was observed that $CaCl_2$ addition significantly enhanced the rate of the mediator-less H_2 production (fig.1). The rate dependence on $CaCl_2$ concentration is rather complicated (fig.2). At the optimal $CaCl_2$ concentration (1.6 mM) the rate of H_2 photoproduction is comparable with that observed in the presence of MV^{2+}, and with the rate of dark H_2 production from MV^{2+} reduced by dithionite (table 1). It should be noted that $CaCl_2$ also activated H_2 production in the case when MV^{2+} was present in the suspension. However, in the presence of MV^{2+}, 1.6 mM $CaCl_2$ caused only 3-fold increase in the reaction rate, while without electron mediator the increase was greater than 30-fold.

Table 1

Hydrogen photoproduction in TiO_2 suspensions

No.	System content	H_2 photoproduction rate ($\mu l \cdot min^{-1} \cdot ml^{-1}$)
1	TiO_2 + hydrogenase	0.05
2	TiO_2 + hydrogenase + MV^{2+} [a]	3.1
3	TiO_2 + hydrogenase + Ca^{2+} [a]	1.7
4	TiO_2 + hydrogenase + Ca^{2+} + MV^{2+}	5.0
5	TiO_2	<0.01
6	TiO_2 + Ca^{2+}	<0.01
7	Dithionite + MV^{2+} + hydrogenase[b]	2.4

[a] MV^{2+} (0.5 mM) and Ca^{2+} (1.6 mM) were added 20 min after irradiation of the system TiO_2 + hydrogenase

[b] Without irradiation

Experimental conditions are given in the text

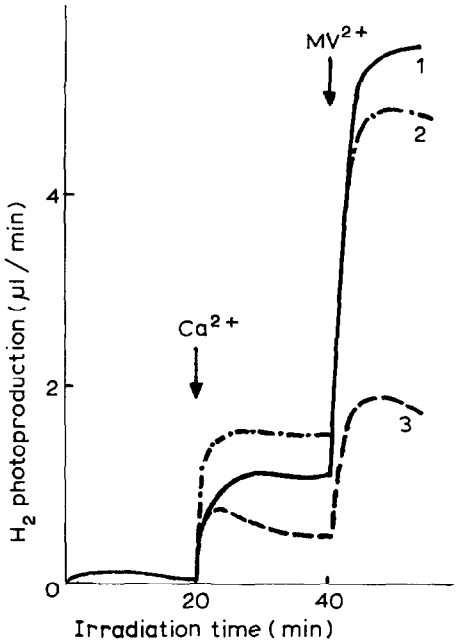

Fig.1. Hydrogen photoproduction in the system Tris-TiO_2 hydrogenase. Arrows indicate additions of $CaCl_2$ (1, 0.4 mM; 2, 1.6 mM; 3, 12.5 mM) and MV^{2+} (0.5 mM).

The dependence of H_2 photoproduction rate on light intensity is non-linear in the system Tris-TiO_2 hydrogenase with 1.6 mM $CaCl_2$ (fig.3). At the light intensity 1×10^3 erg $\cdot cm^{-2} \cdot s^{-1}$ (365 nm) the quantum yield of H_2 photoproduction was shown to be $9 \pm 0.9\%$. It is important that the reaction is not saturated within a broad light intensity range. This testifies to a sufficient electron flow out of the conduction band of the semiconductor to hydrogenase in the absence of electron mediator.

Activation of H_2 photoproduction by $CaCl_2$ was

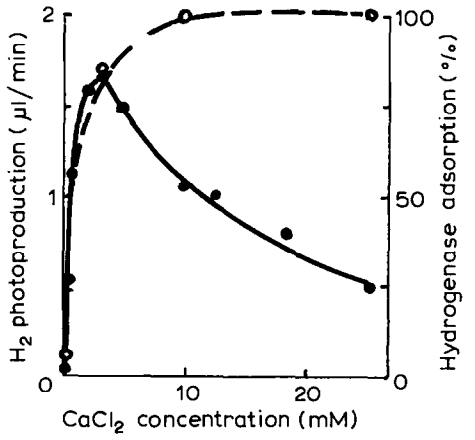

Fig.2. The dependence of hydrogen photoproduction rate (•) and hydrogenase adsorption (○) upon $CaCl_2$ concentration in the system Tris-TiO_2 hydrogenase.

observed not only with Tris buffer as electron donor but with sucrose, or dithiothreitol, or methanol as well, and in the absence of exogenous organic electron donors. In the latter case H_2 photoproduction was significantly lowered and decreased to zero during irradiation. $BaCl_2$, like $CaCl_2$, enhanced the rate of H_2 photoproduction by a factor of about 30. Chlorides of monovalent metals: NaCl, KCl, CsCl at concentrations up to 30 mM increased the reaction rate only 2-fold in the system Tris-TiO_2 hydrogenase.

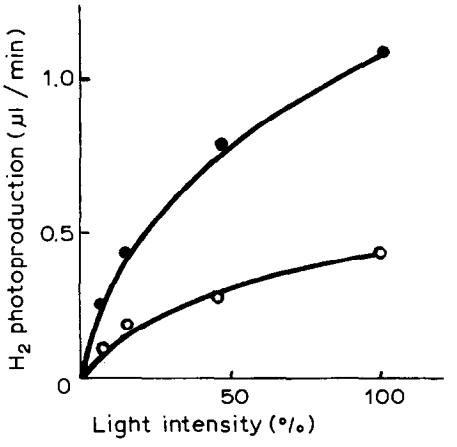

Fig.3. Hydrogen photoproduction in the system Tris-TiO_2 hydrogenase in the presence of 1.6 mM $CaCl_2$ as dependent on light intensity, under irradiation with light of mercury lines 320–800 nm, 100% is 2.8×10^5 erg·cm^{-2}·s^{-1} (•) or 365 nm, 100% is 1×10^4 erg·cm^{-2}·s^{-1} (○).

The data obtained show the activation effect of a salt on H_2 photoproduction to be accounted for by the valency and concentration of the cation and not by changes in the ionic strength.

Experiments using 0.05 M phosphate buffer (pH 7.2) showed the phosphate ions to suppress H_2 photoproduction almost completely in the system consisting of TiO_2, hydrogenase, and 0.5 M sucrose as an electron donor. At the same time, when TiO_2 was coupled with hydrogenase via MV^{2+}, phosphate decreased the rate of H_2 photoproduction only by a factor of 1.5–2.

When a different sample of titanium dioxide P25 TiO_2 (Degussa) was employed in phosphate buffer containing electron donor and hydrogenase, H_2 photoproduction in the absence of MV^{2+} also proceeded at a low rate (<0.01 μl $H_2 \cdot min^{-1} \cdot ml^{-1}$). The difference of this sample from Fluka TiO_2 was manifested when the reaction was conducted in Tris buffer. In the system Trs-P25 TiO_2 hydrogenase a high rate of H_2 photoproduction was achieved (1.0–1.5 μl $H_2 \cdot min^{-1} \cdot ml^{-1}$). MV^{2+} enhanced the reaction rate only 1.5–2-fold, and the addition of $CaCl_2$ led to a small decrease in H_2 photoproduction rate.

It follows from the analysis of the data on hydrogenase adsorption (fig.2, table 2) and on H_2 photoproduction in suspensions of TiO_2 and P25 TiO_2, that the effective H_2 photoproduction in the absence of electron mediator proceeds under the conditions which provide hydrogenase binding with the semiconductor. Indeed, both hydrogenase adsorption on the semiconducting particles (table 2) and the mediator-less H_2 photoproduction are accelerated by Tris addition to water suspensions of TiO_2 or P25 TiO_2 and suppressed in the presence of phosphate. H_2 photoproduction in the absence of electron mediator and hydrogenase binding with semiconductor (fig.2) were similarly enhanced by $CaCl_2$ at concentrations up to 2 mM in TiO_2 suspensions with Tris buffer. Thus, activation of H_2 photoproduction by $CaCl_2$ is connected with its ability to provide hydrogenase adsorption on TiO_2. With the data obtained it is not yet possible to explain the decrease in H_2 photoproduction rate at $CaCl_2$ concentrations higher than 2 mM, when the total of hydrogenase is bound with the semiconductor. Perhaps this is conditioned by coagulation of the semiconducting particles or by the increase in the ionic strength. The increased ef-

Table 2

Adsorption of hydrogenase from *Thiocapsa roseopersicina* on TiO_2 and P25 TiO_2

Medium content	Adsorbed hydrogenase (%)	
	TiO_2	P25 TiO_2
H_2O	1	65
Tris buffer, pH 7.2	6	100
Tris buffer, pH 7.2, $CaCl_2$, 10 mM	100	100
Phosphate buffer, pH 7.2	–	4

ficiency of the mediator-less H_2 photoproduction in P25 TiO_2 suspensions is accounted for by the fact that P25 TiO_2 particles in Tris-buffer adsorb the total of hydrogenase, while TiO_2 particles adsorb only 6% of the hydrogenase.

The ability of TiO_2 to adsorb proteins has been already reported [11,12]. It was also shown that bacterial hydrogenases bound firmly to TiO_2 [7]. The authors made an assumption of hydrogen bond formation between the enzyme and the surface groups of the semiconductor [7]. The data on the properties of TiO_2 surface [13] and the data of the present paper allow one to assume that the binding of hydrogenase from *T. roseopersicina* with TiO_2 in our experiments is conditioned mainly by the electrostatic interactions.

Depending on pH of the solution, the hydrated surface of TiO_2 is charged either positively or negatively due to the amphoteric nature of surface OH groups. The charge of TiO_2 surface is dependent on the sample preparation technique and on the specific adsorption of the ions. The isoelectric point for the purified anatase dispersions is in the pH range 5.0–7.5 [13,14]. Commercial TiO_2 samples have the isoelectric point at pH 3 because of the anion impurities (sulphate, phosphate usually) firmly bound to their surfaces [15]. In our experiments, at pH 7.2 the bivalent cations adsorbed on TiO_2 particles may significantly decrease their negative charge, and, thus, create conditions for binding of hydrogenase whose molecules are negatively charged (p*I* 4.2). Hydrogenase adsorption induced by $CaCl_2$ is reversible. Hydrogenase bound with TiO_2 particles in Tris buffer in the presence of $CaCl_2$ is desorbed on resuspending of TiO_2 sediment in Tris buffer containing no $CaCl_2$. This is in line with the assumption of the electrostatic nature of enzyme-semiconductor interactions. In Tris buffer whose molecules at pH 7.2 are neutral or positively charged, the charge of TiO_2 particles is preserved or becomes more positive. The suppression of hydrogenase adsorption in phosphate buffer may be accounted for by the ability of phosphate anions to bind firmly with TiO_2 surface, increasing the negative charge and occupying the surface centres responsible for hydrogenase adsorption.

The difference in the properties of TiO_2 and P25 TiO_2 may, perhaps, be attributed to the difference in their surface charges. Besides, the highly hydroxylated surface of P25 TiO_2 may have a greater number of the centres available for interactions with protein molecules.

Thus, the data obtained show the possibility of H_2 photoproduction with quantum efficiency up to 10% in the course of direct photoinduced electron transfer from TiO_2 conduction band to the reaction centre of hydrogenase under the conditions providing the enzyme binding to the semiconducting particles.

REFERENCES

[1] Krasnovsky, A.A., Brin, G.P. and Nikandrov, V.V. (1976) Dokl. Akad. Nauk SSSR 229, 990–993.
[2] Krasnovsky, A.A., Brin, G.P., Luganskaya, A.N. and Nikandrov, V.V. (1979) Dokl. Akad. Nauk SSSR 249, 896–899.
[3] Nikandrov, V.V., Brin, G.P. and Krasnovsky, A.A. (1981) Dokl. Akad. Nauk SSSR 256, 1249–1253.
[4] Nikandrov, V.V., Brin, G.P. and Krasnovsky, A.A. (1983) Photobiochem. Photobiophys. 6, 101–107.
[5] Krasnovsky, A.A., Nikandrov, V.V. and Nikiforova, S.A. (1985) Dokl. Akad. Nauk SSSR 285, 1467–1471.
[6] Krasnovsky, A.A. and Nikandrov, V.V. (1987) FEBS Lett. 219, 93–96.
[7] Cuendet, P., Grätzel, M., Rao, K.K. and Hall, D.O. (1984) Photobiochem. Photobiophys. 7, 331–340.
[8] Cuendet, P., Rao, K.K., Grätzel, M. and Hall, D.O. (1986) Biochimie 68, 217–221.
[9] Willner, I., Mandler, D. and Maidan, R. (1987) Nouv. J. Chim. II, 109–121.
[10] Zorin, N.A. and Gogotov, I.N. (1982) Biokhimiya 47, 827–833.
[11] Glueckauf, E. and Patterson, L. (1974) Biochim. Biophys. Acta 351, 57–76.
[12] Messing, R.A. (1976) in: Methods in Enzymology (Mosbach, K. ed.) vol.44, p.148, Academic Press, New York.
[13] Kalyanasundaram, K. (1986) in: Energy Resources through Photochemistry and Catalysis (Grätzel, M. ed.) pp.241–287, Izd. Mir, Moscow.
[14] Healy, T.W. (1965) J. Coll. Sci. 20, 376.
[15] Bobyrenko, Yu.Ya. (1973) Colloid J. 33, 803–806.