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Coupled Action of Cadmium Metal and Hydrogenase in Formate Photodecomposition Sensitized by CdS

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Cadmium metal, CO_2, CO, and H_2 are produced under illumination of deaerated aqueous suspension of CdS in the presence of formate. The mechanism of cadmium metal photoformation and the influence of Cd^0 and the enzyme hydrogenase from *Thiocapsa roseopersicina* on the CdS-sensitized reactions have been studied. The reduction of the surface Cd^{2+} ions of the semiconductor lattice to Cd^0 is the result of the cooperative action of electrons of the conduction band and $CO_2^{•-}$ anion radicals which are the primary products of formate oxidation by semiconductor holes. Up to 20% of Cd^{2+} of CdS can be reduced to Cd^0. Cadmium metal catalyzes the photoformation of molecular hydrogen. An alternative way of conversion of $CO_2^{•-}$ results in the formation of CO and formate dehydration. Immobilized on the semiconductor particles, hydrogenase from *T. roseopersicina* activates processes of photodehydrogenation and photodehydration of formate sensitized by CdS. In the presence of cadmium metal and the enzyme, the rate of hydrogen photoproduction is up to 10 times higher than that catalyzed by Cd^0 alone. Cadmium metal photoformed on the surface of CdS participates in enzyme-catalyzed hydrogen photoproduction in two ways: as an activator of the transfer of the conduction band electrons to the enzyme active sites or as the substrate electron donor in the enzymatic hydrogen production.

Introduction

Enzymes can be used as catalysts in a number of the redox processes photosensitized by inorganic semiconductors. At the present time the processes of hydrogen photoformation, NAD-$(P)^+$ photoreduction, CO_2 photofixation, and photosynthesis of the organic and amino acids catalyzed by enzymes and sensitized by semiconductors have been demonstrated.[1−4] In such systems, an electron generated in the conduction band is utilized by the enzyme for the reduction of the substrate electron acceptor. As a rule, the transfer of electrons from the semiconductor surface to the active center of the enzyme is carried out by the compounds capable of reversible reduction−oxidation (viologens, ruthenium bipyridyl complex, etc.). However, the coupling of semiconductor and enzyme may proceed without an electron carrier due to a direct electron transfer from the particle to the bound protein molecule.[1,3,4] In this case, the efficiency of the photoprocess strongly depends on the effective sorption of the enzyme in a favorable orientation.

A modification of the semiconductor surface by metals may lead to an increase in the efficiency of the photosensitized reactions.[5] Usually metal is applied to the surface of the semiconductor particles by means of photoreduction of ions present in the solution. For sulfides of zinc and cadmium a photoreduction of the lattice ions resulting in the formation of metal on the surface of particles was shown.[6] With CdS this reaction was demonstrated using semiconductor powders[7,8a] and colloids.[8,9] The mechanisms proposed for the reduction of semiconductor lattice ions include sequential[8] or simultaneous[9c] reduction by the conduction band electrons and participation of a highly reductive intermediate, produced in the course of photooxidation of the hole scavenger.[7] Photoproduced cadmium metal catalyzes CdS-photosensitized reactions of hydrogen evolution from water[8] and reactions of two-electron reduction of some organic compounds.[9] We have shown that NAD-

dependent hydrogenase from *Alcaligenes eutrophus* and hydrogenase from *Thiocapsa roseopersicina* are able to use the cadmium metal formed on the CdS surface as a substrate electron donor for reduction of NAD^+ to NADH and molecular hydrogen production, respectively.[10,11] Both enzymes contain iron−sulfur clusters and a nickel ion and belong to the Ni−Fe family of hydrogenases. The nickel ion was shown to be involved in the activation of molecular hydrogen.[12]

Our work is concerned with the mechanism of formate photodecomposition sensitized by CdS. We describe the study of the photoformation of cadmium metal on the surface of CdS particles and the effect of Cd^0 on the CdS-photosensitized formate decomposition under anaerobic conditions. The influence of the enzyme hydrogenase from *T. roseopersicina* on the process has been studied. We show the possibility of the coupled action of two catalysts: cadmium metal and hydrogenase in the semiconductor-sensitized reaction.

Experimental Section

The photochemical experiments were performed in a glass cell thermostated at 25 °C and connected to the line of a gas chromatograph. A stirred suspension of CdS (Aldrich, 99.999%, average particle size 1.5 μm[9a]) was flushed with inert gas and then illuminated with a 500 W halogen lamp. The length of the light path was 5 mm. The radiation of the lamp was filtered through cutoff glass filters, and light in the region 400−600 nm was used. A typical reaction mixture (1 mL) contained 5 mg of CdS, 2 M formate (sodium salt, Sigma), and 0.05 M MES (Reanal) buffered at pH 6.5. (The concentration of MES provided a stable pH in the course of photoreaction.)

The photon flow absorbed by CdS was 8×10^{-8} einstein/s as was determined with ferrioxalate actinometer taking into consideration the absorption spectrum of CdS.

The gaseous phase was periodically flushed into a gas chromatograph column. The column packed with molecular sieves 5A (Serva) was used for hydrogen, oxygen, or CO

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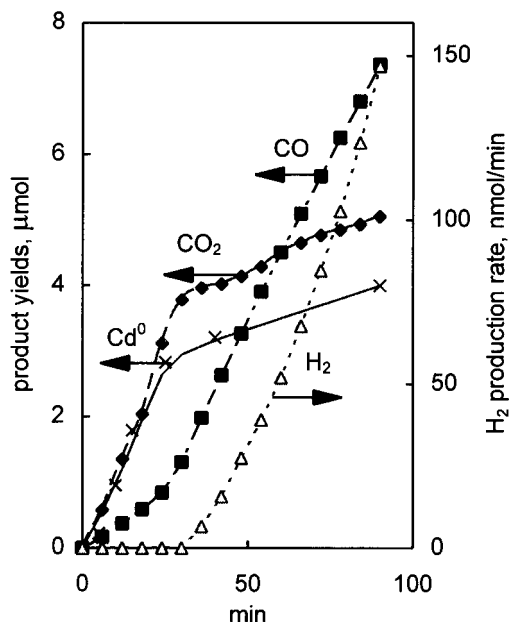
Figure 1. Yields of cadmium metal ($\times$), carbon dioxide ($\blacklozenge$), and carbon monoxide ($\blacksquare$) and the rate of hydrogen evolution ($\triangle$) as functions of illumination time.

analysis (with argon or helium as a carrier gas), and the column with Porapak T was employed for CO_2 determination (with helium as a carrier gas). The kinetics of CO_2 formation were corrected considering the balance of CO_2 in the solution and in the gaseous phase. Addition of the solutions and gases to the cell was made by injections with gas-tight syringes through the rubber septum.

In order to estimate the amount of cadmium metal in a sample, excess oxygen and a small amount (about 10^{-4} M) of methyl viologen were added after illumination was terminated. After a complete oxidation of cadmium-reduced methyl viologen (indicated by the disappearance of blue color belonging to $MV^{\bullet+}$ cation radicals) the amount of oxygen not involved in the reaction was determined, and the amount of cadmium in a sample was calculated according to the stoichiometric equation[11]

$$2Cd^0 + O_2 + 4H^+ = 2Cd^{2+} + 2H_2O$$
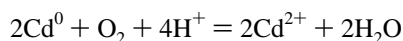
Sulfide anions were detected with the method based on CdS formation in the presence of Cd^{2+} cations.

Hydrogenase from the phototrophic bacteria *T. roseopersicina*, strain BBS, was purified as described previously.[13] When the enzymatic reaction under illumination was studied up to 60 μg of the enzyme was added to the sample before illumination.

The amount of the enzyme bound to the semiconductor surface was evaluated by filtering the sample and measuring the protein content in the filtrate by absorption at 280 nm. The immobilization of enzyme on the semiconductor particles was completed in a few minutes. The formation of the cadmium metal on the surface of the semiconductor did not cause desorption of the proteins.

Results

Formate Photoconversion and Formation of Cadmium Metal in Aqueous Suspensions of CdS. Under illumination of aqueous CdS suspension containing formate in anaerobic conditions, cadmium metal and gaseous products CO_2, CO, and H_2 are formed (Figure 1). Two stages can be distinguished in CdS-photosensitized conversion of formate. The first one is

characterized by the accumulation of cadmium metal on the surface of semiconductor particles. At this stage the maximum rate of carbon dioxide generation is observed while CO generation is relatively small, and hydrogen gas is not formed at all. At the second stage, starting after a certain amount of Cd^{2+} ions of CdS are converted to Cd^0, the rates of metal formation and CO_2 evolution sharply fall, hydrogen generation starts to develop, and the rate of CO production increases sharply. Then, the rates of formation of gaseous products gradually reach stationary values. The maximum achieved rate of H_2 production is much lower than the maximum rates of CO_2 and CO formation. During stationary phase the production of H_2 and CO_2 is almost equal but significantly lower than that of CO. A comparison of the amounts of CO_2 evolved and Cd^0 formed shows that at the first stage of formate photodecomposition they are equal, but when the second stage starts CO_2 production exceeds Cd^0 formation. The addition of CO_2 to the CdS$-$formate system under illumination has no effect on CO production.

The photoformation of Cd^0 in the presence of formate occurs effectively at acidic and neutral pH while no production of Cd^0 is observed at pH higher than 8.5. The rates of photoreactions increase linearly with the formate concentration and reach the broad maximum at concentrations of $2-3$ M. Sulfide anions are not detected in the solution after the reduction of Cd^{2+} ions of the lattice. The initial rate of cadmium metal formation linearly depends on the illumination intensity. The rates of H_2 and CO formation at the stationary state also linearly depend on the light intensity. After the end of illumination the formation of all products stops.

Only a part of Cd^{2+} of the lattice may be reduced to cadmium metal in the photoprocess. With the received CdS powder this amount is about $10-12\%$ of total Cd^{2+} ions. This value can be increased up to 20% if the formate containing suspension of CdS is sonicated before illumination, which extends the available surface of CdS particles. If CdS is sonicated in the presence of EDTA or acetic acid and the supernatant is removed, then nonstoichiometric and weakly bound Cd^{2+} ions are eliminated from the particle surface. The illumination of such CdS preparation in the presence of formate results in a slight decrease in the formed Cd^0 amount compared to the sample sonificated in water; H_2 evolution also decreases, and the period before H_2 evolution becomes shorter.

Assuming that the semiconductor particles are spherical with diameter about 1500 nm, 10% of Cd^{2+} are reduced, and Cd^0 located in the surface layers, we can calculate that the metal layer formed on the surface of CdS is about 50 atom layers thick.

If $CdCl_2$ is added to the CdS$-$formate system, Cd^{2+} cations from solution may be completely reduced under illumination in addition to the reducible ions of the lattice. This is accompanied by evolution of an extra amount of CO_2 equal to the amount of the added Cd^{2+} ions (Figure 2). The initial rate of Cd^0 photoproduction slightly decreases with the increase of Cd^{2+} concentration in the solution. However, if cadmium salt is added to the preilluminated system containing Cd^0, then Cd^{2+} from the solution are converted to metal almost at the same rate as lattice ions.

Addition of $CdCl_2$ to the preilluminated system stops H_2 evolution and significantly decreases CO formation. When all Cd^{2+} ions from the solution are reduced, generation of H_2 and CO increases to the original level. The CdS$-$formate system reduces under illumination such organic oxidants as phenazine methosulfate and flavin mononucleotide. The reduction is accompanied by an intensive CO_2 generation while the rates of
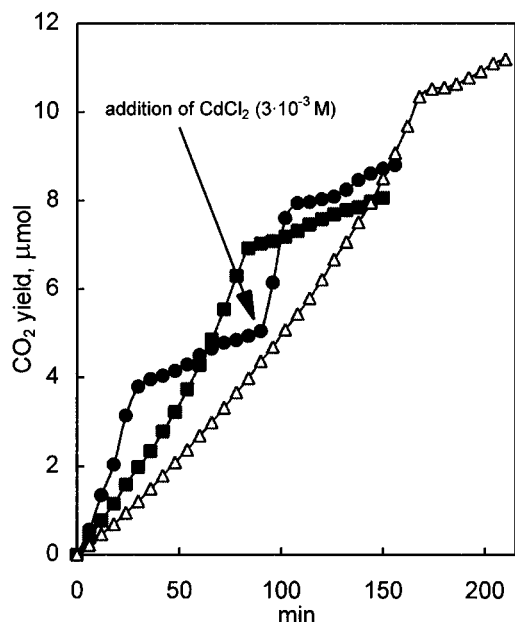
Figure 2. Effect of $CdCl_2$ on the carbon dioxide production. Initial concentrations of $CdCl_2$ are (●) 0, (■) 3×10^{-3}, and (△) 6×10^{-3} M.

H_2 and CO production drastically decrease until the oxidants are completely reduced. Then H_2 and CO formation reaches the normal level.

The addition of methyl viologen (MV^{2+}) to the illuminated system leads to the immediate formation of blue $MV^{•+}$ cation radicals and then to the disappearance of blue color. When added to the preilluminated CdS suspension in the dark, methyl viologen is reduced only to $MV^{•+}$ cation radical. The oxidation of formed Cd^0 with oxygen requires several hours to complete. The presence of methyl viologen accelerates oxidation of the metal with O_2 to a few minutes.

The photoproduction of Cd^0 at lower rates was achieved with sulfite at pH 8 or tartrate as the hole scavengers. Cd^0 photoformation was not observed in the presence of other potential hole scavengers (hydroxy-, amino-, and thio-containing organic compounds, sulfide). If thiol compounds (dithiothreitol, cysteine, mercaptoethanol) efficiently oxidized by CdS were added to the suspension before illumination, no photoformation of Cd^0, CO_2, CO, and H_2 was observed even in the presence of formate. The addition of thiol compounds to the preilluminated CdS−formate system containing Cd^0 resulted in the termination of CO_2 and CO formation and activation of hydrogen photo-evolution.

Effects of Hydrogenase from *T. roseopersicina* on the Processes Taking Place in the Formate−CdS System under Illumination. The addition of hydrogenase from *T. roseopersicina* to the buffered, deaerated CdS suspension containing formate affects the photogeneration of all gaseous products while Cd^0 formation is not altered. The most remarkable changes are observed in the kinetics of hydrogen gas production. Figure 3 shows that H_2 is formed in the formate−CdS−hydrogenase system from the start of the illumination−in contrast to the case when enzyme is absent. During illumination the rate of hydrogen production in the presence of the enzyme grows linearly until a certain point, and then the growth slows down. This point corresponds to the beginning of H_2 generation in the absence of the enzyme when the reducible Cd^{2+} ions of CdS are exhausted.

When all reducible Cd^{2+} ions of CdS are converted to Cd^0 and the formation of cadmium metal ceases, the rate of H_2 photoformation reaches a stable value which may be up to 10
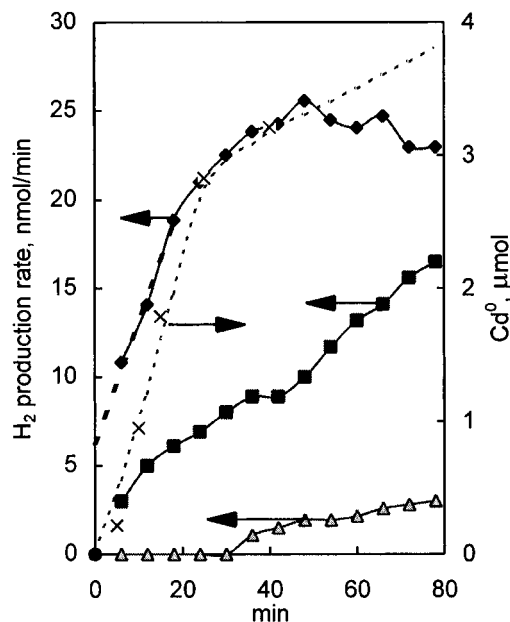
Figure 3. Effect of hydrogenase from *T. roseopersicina* on hydrogen evolution under illumination. Rates of H_2 evolution in the presence of 60 μg of the enzyme (♦) and 30 μg (■) and in its absence (△). (×) shows the yield of cadmium metal.

TABLE 1: Effect of Hydrogenase from *T. roseopersicina* on the Formate Photodecomposition[a]

hydrogenase amount, μg	production rates, nmol/min[b]		quantum yield of formate decomposition[c]
	H_2 (CO_2)	CO	
0	3	90	0.019
60	30	120	0.031

[a] Conditions as in Figure 1, rates were measured after 60 min of illumination. [b] ±5%. [c] Assuming that one photon is required per one hydrogen molecule (see Discussion).

times higher than that in the absence of the enzyme. At this stage the rates of CO and CO_2 evolution become constant as well. The formation of these gases in the stationary phase increases compared to the nonenzymatic system, and CO_2 is formed at the same rate as H_2 (Table 1). Under prolonged illumination, the rate of hydrogen evolution slowly decreases (less than 10% per hour). The decrease is caused by a gradual inactivation of the enzyme while the amount of Cd^0 in the system remains constant.

The cessation of illumination terminates the CO_2 and CO formation while hydrogen evolution continues, in contrast to the nonenzymatic system. H_2 generation is provided by the oxidation of cadmium metal catalyzed by hydrogenase. The kinetic curve of hydrogen formation in the dark represented in Figure 4a reflects several effects. Immediately after the end of illumination the rate of hydrogen production falls followed by some increase before the next slow decrease. The increase is related to the release of CO—a reversible inhibitor of hydrogenase[14]—from the solution. The further decrease in the reaction rate is caused by the exhaustion of Cd^0 due to oxidation by the enzyme.

The amount of hydrogen generated in the enzymatic reaction in the dark is equimolar to that of the oxidized metal. Figure 4b derived from the data of Figure 4a using a known amount of Cd^0 in the system in the end of the dark reaction and its stoichiometry shows that the rate of hydrogen formation is linearly related to the amount of Cd^0 down to a certain metal concentration. The remaining part of the metal is oxidized slowly. In the presence of 10^{-3} M methyl viologen, hydrogenase oxidizes all metal in the sample. The rate of the reaction

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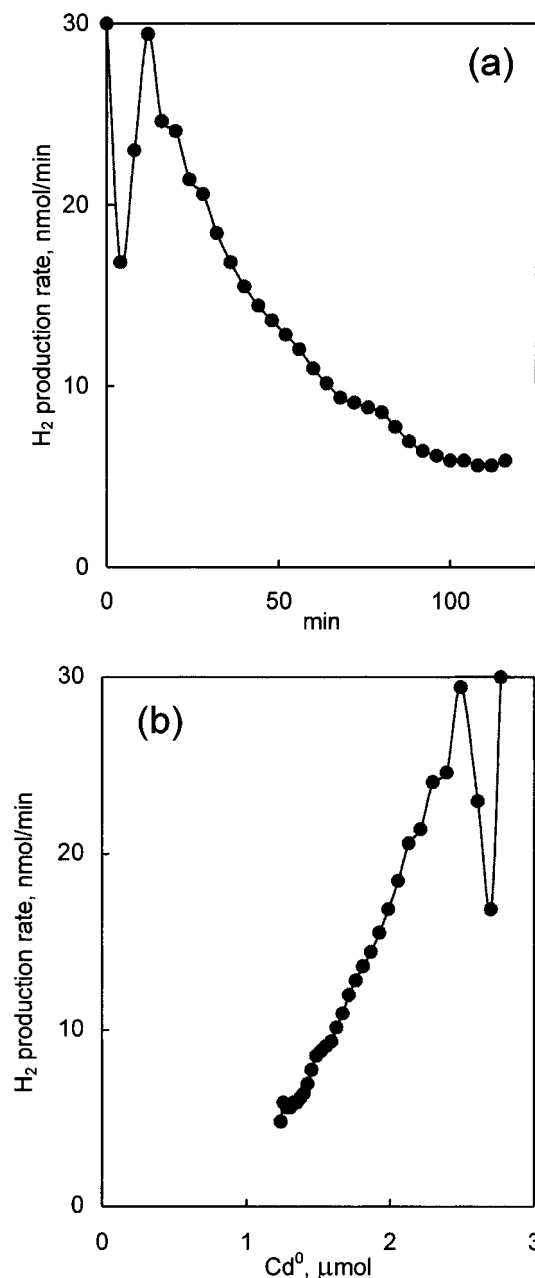
Figure 4. Oxidation of cadmium metal catalyzed by hydrogenase (60 μg). Rates of H_2 production vs time (a) and vs concentration of Cd^0 (b). A total of 2.8×10^{-6} mol of Cd^0 was produced in the course of preillumination of CdS.

is constant (70 nmol of H_2/min with 30 μg of the enzyme) until the amount of remaining metal becomes comparable to that of methyl viologen.

Discussion

The mechanism of cadmium metal photoformation in CdS suspensions containing sulfite as a hole scavenger was discussed previously by Henglein.[8b,c] According to the proposed scheme, semiconductor lattice ions underwent sequential two-electron reduction by electrons of the conduction band, the first electron being trapped and long-lived in the trap:

$$CdS + e_{cb}^- \rightarrow (Cd^{2+}e^-S^{2-})$$

$$(Cd^{2+}e^-S^{2-}) + e_{cb}^- \rightarrow Cd^0 + S^{2-}$$
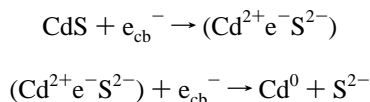
In our experiments the photoformation of cadmium metal strongly depended on the hole scavenger used. The high rate

of Cd^0 photoformation in the presence of formate may be explained by the participation of $CO_2^{\bullet-}$ anion radicals in the reaction. These anion radicals produced as a primary product of the oxidation of formate with holes have strong reductive properties ($E_0 = -2.0$ V). It was shown in pulse radiolysis experiments that $CO_2^{\bullet-}$ anion radicals are able to reduce Cd^{2+} in solution to Cd^+ and to Cd^0.[15] It was earlier assumed that two $CO_2^{\bullet-}$ anion radicals produced at the photooxidation of formate with CdS provide the reduction of Cd^{2+} of the lattice to Cd^0:[7]

$$CdS + 2CO_2^{\bullet-} \rightarrow Cd^0 + S^{2-} + 2CO_2$$
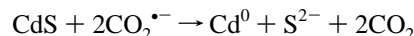
The photogenerated electrons of the conduction band of CdS were proposed to reduce water protons to H_2 in the reaction catalyzed by cadmium metal:

$$H^+ + e^- \rightarrow {}^1/_2H_2$$
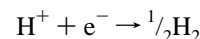
However, we have found that during the first stage of photoreaction when most of the metal is produced, Cd^0 and CO_2 are formed in the ratio 1:1 while no H_2 is evolved (Figure 1). This shows that only one of the two reductive equivalents required for Cd^0 production might be supplied by $CO_2^{\bullet-}$ anion radical. We, therefore, propose that the reduction of the lattice Cd^{2+} of CdS in the presence of formate is the result of the consecutive cooperative action of the trapped electrons and $CO_2^{\bullet-}$ anion radicals:

$$CdS + e_{cb}^- \rightarrow (Cd^{2+}e^-S^{2-})$$

$$(Cd^{2+}e^-S^{2-}) + CO_2^{\bullet-} \rightarrow Cd^0 + CO_2 + S^{2-}$$
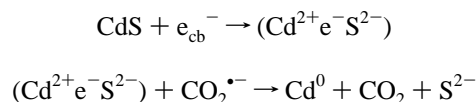
The mechanism may be the same in the presence of other hole scavengers that are able to produce $CO_2^{\bullet-}$ anion radicals under oxidation with holes of the semiconductor. Indeed, we observed the photoreduction of ions in the CdS lattice in the presence of tartrate proceeding at a lower rate than with formate.

The mechanism of Cd^0 formation by simultaneous two-electron transfer was asserted previously for CdS colloids containing the excess of Cd^{2+}, and it was proved by the square dependence of reaction rate on the illumination intensity.[9c] Comparing the potential of CdS conduction band (-0.66 V)[16] with the reduction potential of CdS to Cd^0 (-1.175 V), one may conclude that electrons photogenerated in the semiconductor conduction band cannot reduce the ions of the semiconductor lattice to the cadmium metal. Thus, reduction of nonstoichiometric Cd^{2+} by electrons of the semiconductor conduction band observed earlier[9] may be explained by the fact that the potential of reduction of the excess Cd^{2+} is more positive than that of CdS (E_0 of the pair Cd^{2+}/Cd^0 in solution is -0.4 V) and that the potential of conduction band of CdS colloids is more negative than that of CdS particles. In our experiments reduction of CdS to Cd^0 was up to 20%, and this cannot be explained only by the presence of the nonstoichiometric cadmium in the sample. (This is confirmed by the experiments with CdS powders treated with EDTA or acetic acid.)

Cadmium cations from the solution may adsorb on CdS surface,[17] and the mechanism for their reduction might be similar to that for CdS. But when the surface of the semiconductor particle becomes coated with the metal, two-electron reduction of soluble cadmium cations may occur due to the more positive potential of Cd^{2+} reduction in the absence of S^{2-}.

In accordance with the previously described results,[8] the photoproduction of H_2 is not started until a certain part of Cd^{2+} ions of the lattice is converted to Cd^0 and the catalytic surface
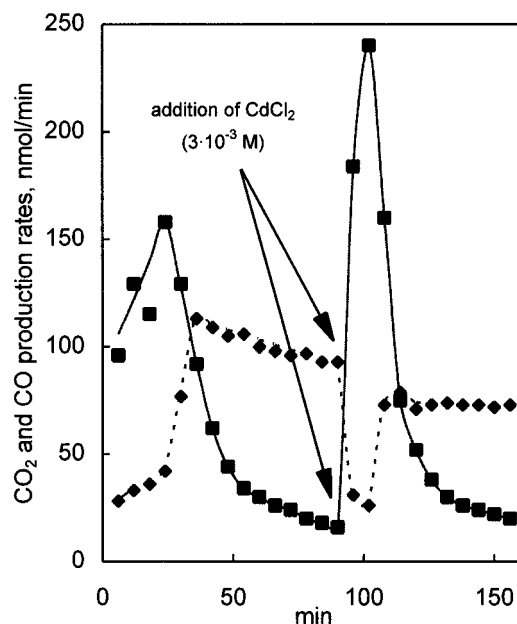
**Figure 5.** Rates of production of carbon dioxide (■) and carbon monoxide (◆) vs illumination time and effect of $CdCl_2$.

Figure 6. Quantum yield of formate photodecomposition as a function of illumination time. Initial concentrations of $CdCl_2$ are (◆) 0, (■) 3 × 10^{-3} M, and (△) 6 × 10^{-3} M.

is formed. Two mechanisms of the metal-catalyzed H_2 production may be proposed. They differ in the way of the participation of $CO_2^{\bullet-}$ anion radicals and in the number of photons required for the formation of a hydrogen molecule. In the first case requiring two photons, $CO_2^{\bullet-}$ may be oxidized with the hole:

$$COOH^- + h^+ \rightarrow CO_2^{\bullet-} + H^+$$

$$CO_2^{\bullet-} + h^+ \rightarrow CO_2$$

$$2H^+ + 2e^- \rightarrow H_2$$
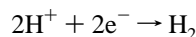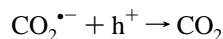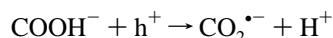
As an alternative, $CO_2^{\bullet-}$ may interact directly with a proton, and in this case one photon is required for production of H_2 molecule:

$$CO_2^{\bullet-} + H^+ \rightarrow CO_2 + {}^1\!/_2H_2$$
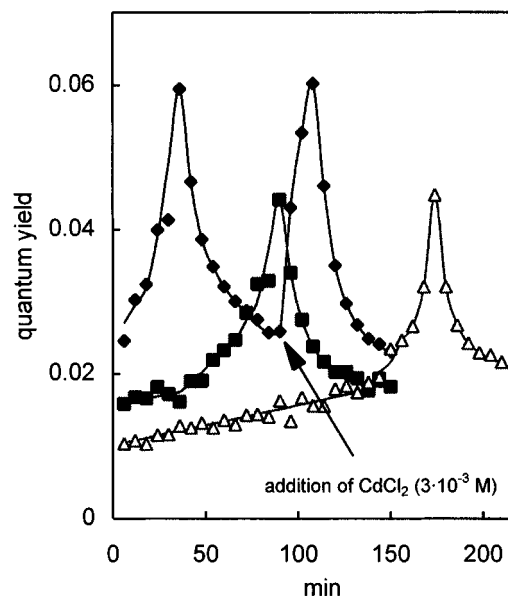
$$H^+ + e^- \rightarrow {}^1\!/_2H_2$$
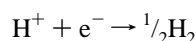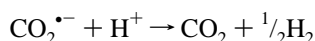
The direct reduction of protons by $CO_2^{\bullet-}$ was shown to be catalyzed by colloidal silver.[18] Only a small part of photogenerated electrons of the conduction band of CdS participates in the H_2 formation even when its rate reaches the maximum. The major part of electrons is involved in the reduction of Cd^{2+} at the first stage of the photoreaction and in the generation of CO at the second stage.

The competition between the processes of CO_2 and CO formation may be suggested from the kinetic curves of photoproduction of these products (Figure 5). Since no reduction of CO_2 to CO sensitized by the semiconductor (demonstrated in other systems[19]) was observed in our experiments, it may be assumed that CO formation is the alternative way of the conversion of $CO_2^{\bullet-}$ anion radicals interaction with the electrons of the conduction band:

$$CO_2^{\bullet-} + H^+ + e^- \rightarrow CO + OH^-$$
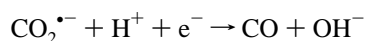
This reaction was proposed to occur during photoelectrochemical reduction of CO_2.[20] The growth of CO formation at

the second stage of the photoreaction is caused by an increase in the amount of participating electrons that were employed in Cd^0 formation at the first stage. The kinetics presented in Figure 1 show that the yield of CO_2 is equal to the sum of the Cd^0 and H_2 productions. It indicates that dimerization of $CO_2^{\bullet-}$ anion-radicals that proceeds with diffusion-controlled rate in aqueous solutions $(2k = 1.5 \times 10^9 \, M^{-1} \, s^{-1})^{21}$ does not occur in our experiments. Perhaps, it is related to the efficient adsorption of $CO_2^{\bullet-}$ anion radicals on the semiconductor particles as it was proposed previously for ZnS.[22] Taking into consideration the described mechanisms, the rates of the consumption of the charge carriers photogenerated in the semiconductor in chemical reactions may be expressed via the rates of the product formation as follows:

$$\left(\frac{d[e]}{dt}\right)_r = \frac{d[Cd^0]}{dt} + n_1\frac{d[H_2]}{dt} + \frac{d[CO]}{dt}$$

$$\left(\frac{d[h]}{dt}\right)_r = n_2\frac{d[CO_2]}{dt} + \frac{d[CO]}{dt}$$

where n_1 and n_2 are numbers of photons required for the formation of H_2 and CO_2 molecule, respectively, n_1 and n_2 values being from 1 to 2. The quantum yield of formate photodecomposition (the ratio of the number of photogenerated electron−hole pairs involved in the redox reactions to the number of the absorbed photons), may be expressed as

$$\phi_{eh} = \frac{\left(\dfrac{d[eh]}{dt}\right)_r}{dN_p/dt} = \frac{n_2\dfrac{d[CO_2]}{dt} + \dfrac{d[CO]}{dt}}{dN_p/dt} =$$

$$\frac{n_1\dfrac{d[H_2]}{dt} + \dfrac{d[CO]}{dt} + \dfrac{d[Cd]}{dt}}{dN_p/dt}$$

where dN/dt is the number of photons absorbed by the semiconductor per unit of time.

Figure 6 shows the changes of the quantum yield during illumination and the effect of the addition of nonstoichiometric Cd^{2+} ions. Since the production of H_2 is low as compared to the other products, the value of n_1, which may range from 1 to

2, almost does not effect the dependence curves in Figure 6. The quantum yield achieves the maximum value at the end of the first and the start of the second stage of the formate photoconversion.

It is possible that the growth of the quantum yield at the first stage is related to an increase of the formate photooxidation rate as a result of a change of a surface charge of the CdS particles following the reduction of Cd^{2+}. It is known that Cd^{2+} ions adsorbed on the semiconductor surface shift the flat-band potential to the positive side hampering the release of electrons from the semiconductor.[17,23] If the rate of CdS-photosensitized reaction is determined by the rate of electron acceptor reduction, then the reduction of the ions of CdS lattice leading to the shift of the flat-band potential to the negative side should enhance ϕ_{eh}, while the addition of $CdCl_2$ should produce the opposite effect. As it is shown in Figure 6, the addition of $CdCl_2$ to the aqueous suspension of non-preilluminated CdS decreases the quantum yield which grows in the course of the reduction of Cd^{2+}. This allows us to assume that the process of electron acceptor reduction is rate determinant of the first stage of the photoreaction. The same is true for the second stage, when the quantum yield of formate photodecomposition is gradually decreased to the stationary value. This may be suggested from the increase of ϕ_{eh}, when some electron acceptor (e.g., $CdCl_2$) is added to the system at this stage (Figure 6). The difference between the rates of excess Cd^{2+} reduction at different stages of the photoreaction shows the catalytic role of the accumulated cadmium metal in the process of excess Cd^{2+} photoreduction.

Thus, suspension of CdS under illumination sensitizes the reactions of formate photodehydrogenation resulting in the production of CO_2 and formate photodehydration leading to the formation of CO. The reactions coupled with the formate photodehydrogenation are the reduction of Cd^{2+} at the surface of semiconductor particles to Cd^0 and the reduction of water protons to H_2. Hydrogen formation is catalyzed by Cd^0 accumulated on the surface of CdS particles.

Experimental results show that the hydrogenase from *T. roseopersicina*, which is the catalyst of H_2 production, increases the rates of the formation of gaseous products of formate photodecomposition, which results in the enhanced quantum yield of the photoreaction (Table 1). The activation of formate decomposition with hydrogenase occurs due to the acceleration of the transfer of conduction band electrons to H^+. This is supporting evidence in favor of electron acceptor reduction being the rate-limiting step. The acceleration of H^+ reduction consequently causes the increase of the rate of formation of $CO_2^{\bullet-}$ anion radicals. The additionally produced $CO_2^{\bullet-}$ radicals could not have been reduced to form CO, because the enzyme is unable to catalyze the reaction. Therefore, the growth of CO production observed in the presence of the enzyme makes it possible to assume that $CO_2^{\bullet-}$ radicals react with each other:

$$2CO_2^{\bullet-} + H^+ \rightarrow CO_2 + CO + OH^-$$
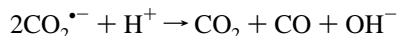
Almost equal absolute values of the increase in CO and CO_2 rates in the presence of hydrogenase (Table 1) testify to the functioning of the mechanism described.
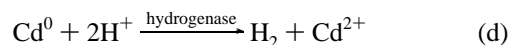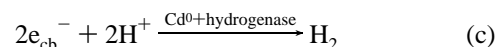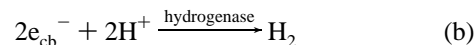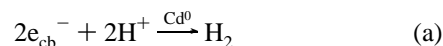
It was demonstrated earlier[3b] that the hydrogenase is able to catalyze hydrogen evolution under a direct transfer of electrons of the conduction band of CdS or TiO_2. In the formate−CdS−Cd^0−hydrogenase system photoformation of H_2 may be catalyzed by the metal, or the enzyme, or due to the coupled action of these catalysts.

The increase of the rate of hydrogen production during the accumulation of cadmium metal in the course of illumination (Figure 3) shows that photoformed cadmium metal affects the enzymatic reaction. At the beginning of illumination the dependence between the metal amount and H_2 rate is close to linear. With the linear extrapolation of the kinetic curve of hydrogen photoevolution rate in the presence of hydrogenase (Figure 3) to the time of the onset of illumination (when no Cd^0 is formed yet) gives the value of H_2 formation rate in the absence of metal, 6 nmol/min. The rate of H_2 formation at the stationary phase reaches 30 nmol/min. This is 3 times higher than the value which could be expected if the hydrogen production were catalyzed independently by the metal and the enzyme.

Cadmium metal may affect enzymatic hydrogen photoformation (i) activating the transfer of the conduction band electrons to the enzyme active sites that may be related to the acceleration of the transfer of the electrons generated in the conduction band of CdS to hydrogenase active sites or to the alteration of the orientation of immobilized hydrogenase following the modification of the surface and (ii) Cd^0 being a substrate of the enzymatic reaction, i.e., being used by hydrogenase as the electron donor in the molecular hydrogen formation with the following regeneration due to formate photodecomposition.

Thus, the following ways of molecular hydrogen photoformation in the system formate−CdS−hydrogenase from *T. roseopersicina* may be

$$2e_{cb}^- + 2H^+ \xrightarrow{Cd^0} H_2 \qquad (a)$$

$$2e_{cb}^- + 2H^+ \xrightarrow{hydrogenase} H_2 \qquad (b)$$

$$2e_{cb}^- + 2H^+ \xrightarrow{Cd^0+hydrogenase} H_2 \qquad (c)$$

$$Cd^0 + 2H^+ \xrightarrow{hydrogenase} H_2 + Cd^{2+} \qquad (d)$$

The total rate of hydrogen production under illumination may be presented as the sum of these four processes. The contribution of each process to hydrogen production in the stationary phase when concentration of Cd^0 remains constant may be roughly estimated considering the change in H_2 evolution after the end of illumination. Figure 4a shows that during the first minutes after the end of illumination the rate of hydrogen production falls. This decrease is described by three first reactions listed above. The linear extrapolation of the further increase (due to removal of CO) in the kinetic curve of hydrogen production to the time of the end of illumination gives the value for the rate (d) 11 nmol/min. Thus, the sum of rates (a), (b), and (c) becomes equal to 19 nmol/min. The rate of the nonenzymatic process (a) cannot exceed the value of 3 nmol/min that it has in the absence of the enzyme (Table 1). The stationary value of (b) seems to be negligible, because at the stationary phase the semiconductor particles become coated by the metal. Thus, the processes (c) and (d), reflecting the transfer of the electrons generated in the conduction band across the metal to the enzyme and the enzyme-catalyzed oxidation of the metal, respectively, are two major contributors to hydrogen production under illumination. It is worth mentioning that platinized CdS particles were not found to be better catalysts for the formate photodecomposition compared with those coated by cadmium metal.[7] It should be noted that in processes (a)−(c) one of two electrons required for the formation of the hydrogen molecule may be provided by $CO_2^{\bullet-}$ anion radical.

Figure 7 summarizes the reactions proceeding under illumination of CdS suspensions containing formate, Cd^0, and hydrogenase. Thus, metal and enzyme can act jointly as catalysts of
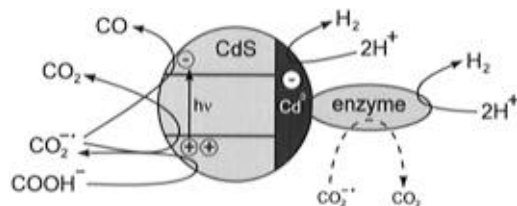
Figure 7. Pictorial diagram illustrating some of the reactions taking place in the course of the formate photodecomposition, sensitized by CdS and catalyzed by hydrogenase.

the photoprocesses sensitized by semiconductors. The coupled action of metal and enzyme is provided with the participation of the metal as the carrier of electrons of the semiconductor conduction band to the enzyme active sites bound with the surface of the semiconductor particles and by the enzyme-catalyzed oxidation of the metal accumulated during illumination of the semiconductor.

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