Reduction of CO_2 by *Halobacterium Halobium* MMT_{22} to Formic Acid

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Photobiological and photoelectrochemical conversion of CO_2 to formic acid was achieved by using *Halobacterium halobium* MMT_{22}. The yield of formic acid increased considerably by regenerating NADH by cathodic reduction at −0.60 V or in the presence of the two-electron donor ascorbic acid. Under photoelectrochemical reduction, maximum yield of 0.2 mol of HCOOH/mg cells/h was obtained, the highest reported so far.

With the variety of industrial processes, large amounts of CO_2 are released into the atmosphere causing pollution and ecological problems.[1] CO_2 is widely used for large scale preparation of urea and certain inorganic chemicals such as carbonates and hydrogen carbonates.[2] Carbon dioxide being the abundant source of carbon, wide options are available for converting this valuable C_1 synthetic block into industrial organic chemicals.

There are numerous reports on the microbial-Autotrophic and Heterotrophic carbon dioxide fixation.[3] In *Halobacterium halobium,* photoactivation of the bacteriorhodopsin results in the transfer of protons from the interior to the exterior of the cells. It is now generally believed that 2—3 H^+ are translocated across the purple membrane per cycling bacteriorhodopsin.[4]

Under anaerobic conditions in the light *H. halobium* synthesizes ATP[5] and carbon uptake is greatly increased. Carbon dioxide is fixed through reductive pathways and NADH is produced during illumination by reversal of electron transport.[6] This reduced nicotinamideadenine dinucleotide (NADH) functions as an electron donor in the biological CO_2 fixation.[7] Photochemical reduction of CO_2 to formic acid in the presence of $[Ru(bpy)_3]^{2+}$ and $[Ru(bpy)_2(CO)_2]^{2+}$ (bpy=2,2′-bipyridine) was reported in H_2O/DMF in the presence of NADH model compound 1-benzyl-1,4-dihydronicotinamide (BNAH) or triethanolamine/*N,N*-dimethylformamide system.[8,9]

The NADH/NAD^+ complex was regenerated both by constant potential coulometry at −0.60 V and by the use of a two-electron donor ascorbic acid. The efficiency of the system increases in the order photochemical<photochemical+ascorbic acid<photoelectrochemical. Recently, cyclohexanone was reduced to cyclohexanol by coupling NADH/NAD^+ coupled to alcohol dehydrogenase. The two electrons needed to regenerate NADH is provided electrochemically at −0.73 V vs. Ag/AgCl. In the photochemical or the photoelectrochemical systems, there is a need to transfer two electrons and a proton to NAD^+ to make the system catalytic. The transfer is mediated by a hydride-transfer agent usually a rhodium(I) complex that goes through Rh^I/Rh^{III} redox change by hydride transfer.[9] In the absence of electrons catalysis, a sacrificial two-electron donor such as triethanolamine or EDTA is needed to complete the catalytic cycle.[7,8]

Experimental

The extreme halophile, *H. halobium* MMT_{22} was isolated from the brine obtained from the Salt Farm of CSMCRI in a nutrient medium at pH 7.0. Incubation was carried out at 40 °C under illumination.

Photobiological studies were carried out in a 50 ml reaction vessel maintained at 40 °C through a thermostatic water bath. 1 mg (wet weight) of *H. halobium* MMT_{22} was taken in an air tight vessel containing 35 ml of the above nutrient medium and in a solution containing 35 ml of 25% NaCl, respectively. The O_2-free CO_2 gas was supplied to the reaction vessel under illumination with a light intensity of 0.3 mW cm^{-2}.

In a second set of experiment, 1 mg ascorbic acid was added to the reaction medium.

5 ml of the sample was taken out every 2 hours interval and made free of cells by centrifugation. The cell-free suspension was then subjected to distillation on steambath so as to make the products free of the very high sodium chloride concentration.

Controlled potential coulometric electrolysis was carried out with a PAR Model 173 potentiostat and Model 179 current integrator. A coulometric cell of three electrode configuration consisting of mercury as working electrode, platinum as counter electrode and calomel as reference electrode was used. Deaeration of the solution consisting of 12 ml mercury and 35 ml 0.1 M tetraethylammonium chloride (1 M=1 mol dm^{-3}) was done by passing N_2 through the solution for 15 min in a coulometric cell of 50 ml. Oxygen present in the system was reduced at −0.9 V. After observing very small background current for blank, 1 mg (wet weight) of *H. halobium* MMT_{22} was introduced in the reaction vessel separately and CO_2 gas was passed. The reaction vessel was illuminated with a light intensity of 0.3 mW cm^{-2}. The rate of CO_2 reduction by electrolysis at Hg cathode at −0.6 V vs. SCE was measured by taking out sample at every 2 hours interval, followed by separation of cells by centrifugation and distillation. Control experiments were done in a similar system devoid of cells at −0.6 V vs. SCE when CO_2 was supplied.

The products were analyzed using a high-performance liquid chromatograph, Water Model 501 with automated

Gradient Controller, SE 120 manual chart recorder, model 740 data module, Model U6k as sample injecting system, Model 430 as conductivity detector (expressed in μS) with anion (A) column using borate buffer as an eluent solution. Flow rate was kept at 1 ml min^{-1}.

Results and Discussion

Under strictly anaerobic conditions on illumination, each bacteriorhodopsin cycling in the purple membrane of *H. halobium*, releases 2—3 protons which are released on the exterior of the cells. Simultaneously carbon uptake is greatly increased as CO_2 is fixed through the reductive pathways and NADH is produced during illumination by reversal of electron transport. Thus, NADH functions as an electron donor in the system.

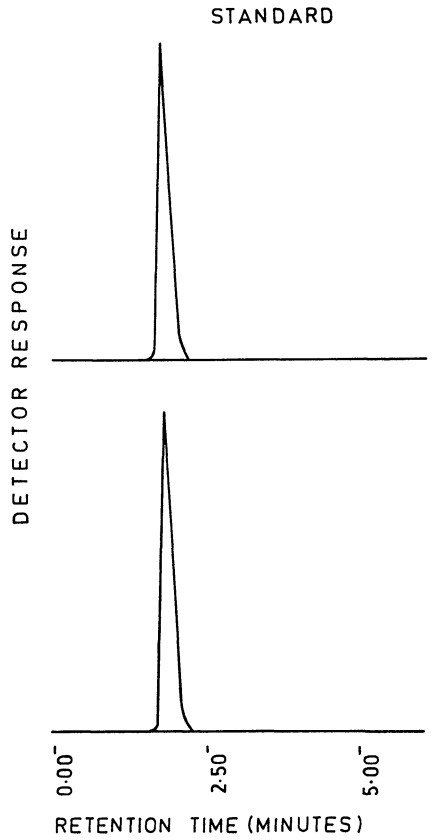

Fig. 1. Selective formation of formic acid by *H. halobium* MMT_{22} as analyzed by HPLC.

As shown in Fig. 1, *H. halobium* MMT_{22} gave a selective production of formic acid with no other by-products. The probable products of carbon dioxide reduction such as methanol, formaldehyde, formic acid were checked with the relevant standards on Shimadzu Gas Chromatograph Model GC-9A qualitatively. Formic acid was detected as the sole product. Further, quantitative studies were done on HPLC because of its higher sensitivity by making standard concentration of formic acid and matching the results with its.

The solubility of carbon dioxide in water at 26 °C is 0.0320 M which means 0.0320 M formic acid should be produced after 100% conversion in time *t*. We, however, obtained 1.25 M of formic acid after 6 hours which is almost 40 times of the equilibrium solubillty concentration of CO_2 because of continuous bubbling of the gas through the reaction medium. This results in the accumulation of formic acid in the reaction vessel. The yield can, therefore, be considered as

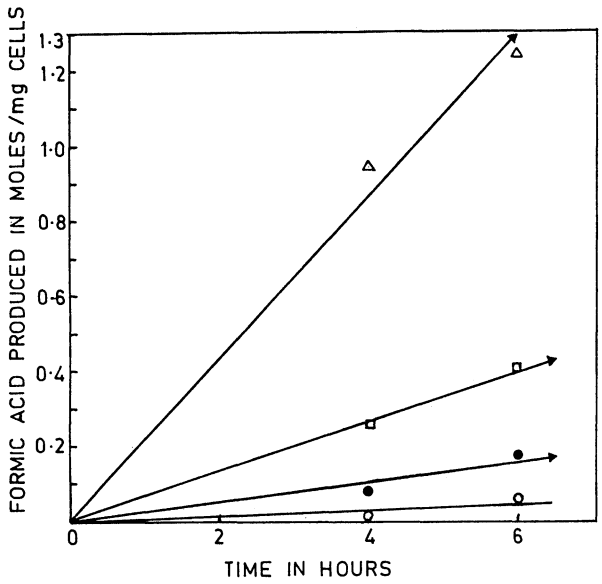

Fig. 2. Reduction of CO_2 to formic acid by *H. halobium* MMT_{22}. O—O: Photobiological formic acid production in 25% NaCl. ●—●: Photobiological formic acid production in nutrient medium. □—□: Effect of ascorbic acid on photobiological in nutrient medium. △—△: Photoelectrochemical formic acid production at −0.6 V vs. SCE.

Table 1. Reduction of CO_2 to Formic Acid by *H. halobium* MMT_{22}

Time	Formic acid produced/mol/mg cells			
	Photobiological			Photoelectrochemical
h	*H. halobium* MMT_{22} in 25% NaCl	*H. halobium* MMT_{22} in nutrient salt medium	*H. halobium* MMT_{22} +ascorbic acid	*H. halobium* MMT_{22} at −0.6 V vs. SCE
0	—	—	—	—
4	0.002	0.098	0.26	0.95
6	0.005	0.182	0.41	1.25

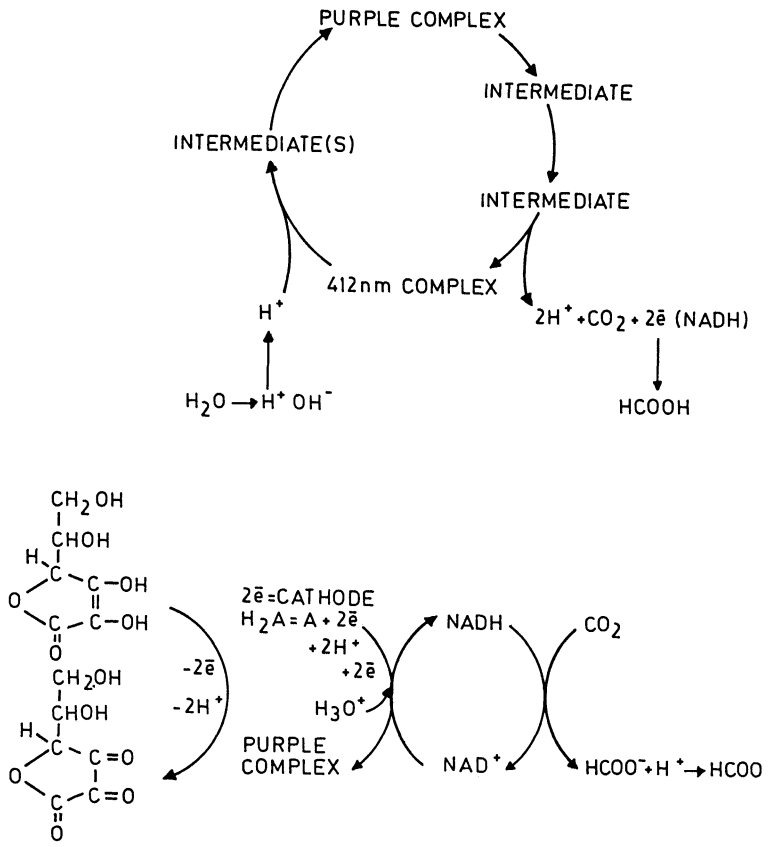

Fig. 3. Mechanism for reduction of CO_2 by *Halobacterium halobium* MMT_{22} to formic acid.

100% with a turnover frequency of 0.20 mol of formic acid per mg cells per hour.

As shown in Table 1, the cells when suspended in a nutrient medium show enhanced yield of formic acid production as compared to those when suspended in a solution containing 25% NaCl. Cells suspended in distilled water do not give favorable results since in the absence of NaCl, the cells burst and the cellular constituents come out in the medium. Photobiologically, reduction of CO_2 to formic acid is a continuous process as long as the cells are kept under strictly anaerobic conditions with proper supply of nutrients and illumination.

When 1 mg ascorbic acid was added in the above system, almost 2.2 times increase in the formic acid production was observed. Ascorbic acid serves as a two-electron donor which thereby enhances the rate of CO_2 reduction by maintaining the $NADH/NAD^+$ cycle.

Seven times increase in the formic acid production was obtained photoelectrochemically at −0.6 vs. SCE using *H. halobium* MMT_{22} as compared to the photobiological experiments and three times when compared to the experiments in the presence of ascorbic acids. This shows the best quantum yields of formic acid formation reported so far (Fig. 2). CO_2 reduction to formic acid photoelectrochemically using *H. halobium* MMT_{22} is also a continuous process.

Reports on electrochemical and photochemical CO_2 reduction catalyzed by metal complexes are many, but, none of them shows more than 2.7% yield of the formic acid production. The maximum yield of HCOOH produced in 10 hours is reported to be 20 μmoles photochemically as compared to *H. halobium* which shows continuous production of HCOOH at the rate of 0.2 mol/mg cells/hour. The system does not need a hydride carrier such as Rh(I) complex and is capable of directly transferring $e^- + H^+$ (H^-) to the $NADH/NAD^+$ couple. The mechanism of the reaction is shown in Fig 3.

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