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The Reversible Hydrogenase in *Anacystis nidulans* Is a Component of the Cytoplasmic Membrane

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Hydrogen metabolism of cyanobacteria has recently attracted special attention because these organisms are potential sources for the bioconversion of light energy to molecular hydrogen [1]. In photoautotrophically growing cyanobacteria, H_2 production comes exclusively from nitrogenases catalyzing the reaction $8H^+ + 8e^- + N_2 \rightarrow 2NH_3 + H_2$ [2]. The cyanobacterium *Anabaena variabilis* can express three different types of nitrogenases, the conventional Mo-enzyme or the two alternative nitrogenases containing either V or only Fe-S centers in the prosthetic group [3, 4]. The alternative nitrogenases are more active in the photoproduction of H_2 than the Mo-enzyme. On the whole, however, intact cells of photoautotrophically growing cyanobacteria produce rather small amounts of the gas. This is due to the fact that the H_2 produced is immediately reutilized by hydrogenases catalyzing the reaction $H_2 \leftrightarrows 2H^+ + 2e^-$.

After a period of controversy it is now established that cyanobacteria possess two different hydrogenases [5]. One enzyme, the so-called uptake hydrogenase, is found in the membrane fraction when cyanobacterial cells are disrupted. It is involved in the light-stimulated consumption of H_2 in intact cells of *Nostoc* PCC 73102 [6] or in the photoreduction of $NADP^+$ by thylakoid preparations from *Nostoc muscorum* (= *Anabaena* 7119) [7]. The enyzme, when either bound to membranes or in the isolated state, virtually does not catalyze H_2 evolution. All the evidence suggests that the uptake hydrogenase is a component of the thylakoid membranes. It is particularly active in heterocysts of free-living cyanobacteria [8]. A recent immunological study with antibodies against the uptake hydrogenase from *Alcaligenes latus*, however, showed that the enzyme is more or less evenly distributed in the cytoplasm of both heterocysts and vegetative cells of the symbiotic *Nostoc* PCC 73102 isolated from the roots of the cycad *Macrozamia* [6]. The other enzyme, which catalyzes both the

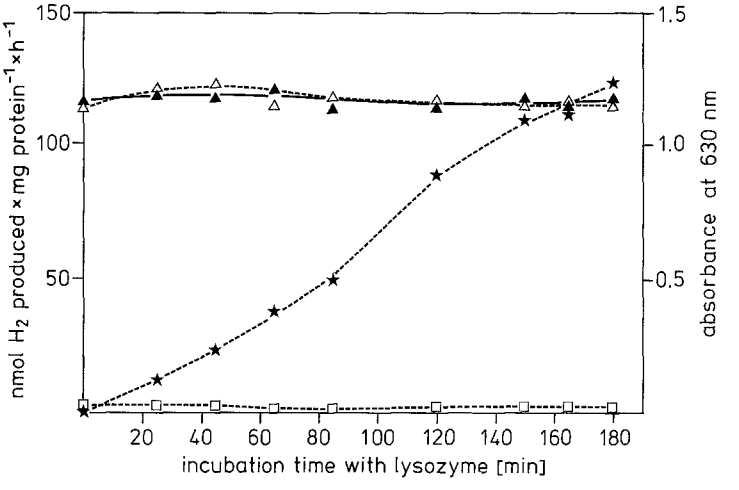

Fig. 1. The activity of the reversible hydrogenase from *Anacystis nidulans* in the membrane fraction. *A. nidulans* cells from 1.5 l culture in the logarithmic growth phase were centrifuged (10 min, 4000 *g*). The cells were resuspended in 40 ml buffer containing 10 m*M* TES pH 7.0, 2 m*M* EDTA, 0.25 *M* sucrose, 0.2% lysozyme. The suspension was incubated at 40 °C in a shaking water bath and aliquots were removed at different times as indicated. After centrifugation of the aliquots at 8000 *g* the pellets were resuspended in 20 m*M* TES pH 7.0, 0.2 *M* sucrose. The resuspended cells and the supernatants were tested for $Na_2S_2O_4$/methylviologen-dependent H_2 evolution (see [15]). Activity: ▲ in the *Anacystis* suspension (control without centrifugation), △ after centrifugation in the pellet, □ after centrifugation in the supernatant, ★ absorbance at 630 nm in the supernatant due to phycobiliprotein release

uptake and the evolution of H_2 in vitro (= reversible or bidirectional hydrogenase), is readily found in the supernatant when cells are disrupted and centrifuged and is, therefore, considered to be a soluble enzyme in cyanobacteria [8]. Immunolocalization using polyclonal antibodies raised against this enzyme from *Anacystis nidulans* indicated that it occurs in the vicinity of the cytoplasmic membrane [5]. The gold-labeling, however, was coarse and therefore did not allow one to conclude that the enzyme is a component of the cytoplasmic membrane, or that it belongs to the cytoplasm or to the periplasmic space.

Lysozyme treatment of *Anacystis* cells causes a gradual degradation of the peptidoglycan layer followed by a lysis of the cells. This can be monitored by the increase in absorbance at 630 nm which is due to the release of phycobilins out of the cells (Fig. 1). During this treatment, the activity of the reversible hydrogenase (determined by the $Na_2S_2O_4$ and methylviologen-dependent H_2 evolution) stays completely in the pellet. Figure 1 also shows that centrifugation and resolubilization of the pellet does not destroy the activity of the reversible hydrogenase at all. The enzyme is readily solubilized and its activity drastically increases when the pellet is treated with low concentrations of the detergent Triton-X-100 (Fig. 2).

These findings indicate that the reversible hydrogenase is membrane-associated, probably as a peripheral and not as an integral protein. The immunolocalization experiments reported earlier [5] had shown that this structure must be the cytoplasmic membrane, as no labeling had been found at the thylakoids. The enzyme might be bound to the cytoplasmic membrane by hydrophobic interactions which can easily be destroyed by treatments like sonication, breaking the cells in a French press, or washing with detergents. In this respect the reversible hydrogenase is not unique, as a similar behavior had been reported for the coenzyme F_{420}-reducing hydrogenase from *Methanosarcina barkeri* [9, 10] and one of the hydrogenases from *Alcaligenes eutrophus* had been found to reversibly detach from the cytoplasmic membrane [11].

The low apparent K_m of the reversible hydrogenase in cyanobacteria for H_2 suggests that it functions as a H_2-utilizing enzyme also. It might reside at the periplasmic face of the cytoplasmic membrane as the H^+ gradient is directed outwards in cyanobacteria. This membrane was shown to contain components of the respiratory chain like quinones, the cytochrome b_6/f complex, and the cytochrome oxidase [12, 13]. Recently, an NADH-dehydrogenase homologous to the mitochondrial complex I was demonstrated for the cytoplasmic and thylakoid membranes [14]. The current findings add the reversible hydrogenase to the components of an electron transport chain of the cytoplasmic membrane. It is likely that such an electron transport results in the generation of a protonmotive force and ATP synthesis. The generation of a H^+ gradient from H_2 or NADH has not been demonstrated as yet for the cytoplasmic membrane of cyanobacteria. The interaction between the respiratory chains on both thylakoids and the cytoplasmic membrane and the photosynthetic electron transport of thylakoids also remains an intriguing question for cyanobacteria.

This work was kindly supported by a grant from the BMFT (no. 9342 A/H 2-01-04-90 program "Biologische Wasserstoffgewinnung").

Received October 2, 1991

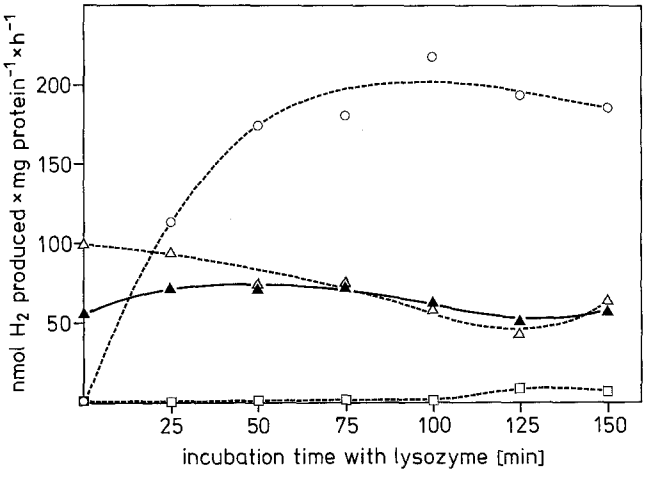

Fig. 2. Solubilization of the reversible hydrogenase by incubation with lysozyme. The experiments were performed as for Fig. 1. In addition, the pellets were resuspended in 20 m*M* TES pH 7.0, 0.05 % Triton-X-100 and recentrifuged at 8000 *g*. The supernatants were tested for H_2 evolution. Activity: ▲ in the *Anacystis* suspension (control without centrifugation), △ after centrifugation in the pellet, □ after centrifugation in the supernatant, ○ in the supernatant after washing the pellet with Triton-X-100

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