The effects of acetaldehyde on nitrogenase, hydrogenase and photosynthesis in the cyanobacterium *Anabaena cylindrica*

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Acetaldehyde was shown to be an irreversible inhibitor of nitrogenase, hydrogenase, CO₂ fixation and growth in the cyanobacterium *Anabaena cylindrica*, but had no effect on photosynthetic electron flow as measured by Methyl Viologen-dependent O₂ uptake. The concentration-dependence of the inhibition of nitrogenase and hydrogenase activities was determined, and it was shown that acetaldehyde inhibition poses problems for anaerobic experiments in which the activities of these enzymes are measured in the presence of the frequently used glucose/glucose oxidase/catalase/ethanol O₂ trap. It is suggested that acetaldehyde may find use as an inhibitor in experiments designed to separate electron flow through the photosystems from consequent fixation of CO₂ and N₂.

The following O₂ trap is frequently used in studies of photosynthetic systems such as biophotolysis with chloroplast/hydrogenase mixtures (Rao & Hall, 1979):

In using this O₂ trap to study the effects of oxygen on nitrogenase in *Anabaena cylindrica*, we have found that the acetaldehyde derived from photosynthetically produced O₂ was inhibitory to both nitrogenase and hydrogenase activities in this organism. The present paper describes the nature of the inhibition, together with the effects of acetaldehyde on electron transport and CO₂ fixation.

Methods

Cyanobacteria and their growth

Anabaena cylindrica (strain 29414) was obtained from the American Type Culture Collection and grown as described previously (Daday *et al.*, 1977), but with the growth medium at one-eighth strength with respect to all components except phosphate. Cells were harvested, by centrifugation, at a concentration of approx. 100 Klett units (Mallette, 1969) and concentrated, by centrifugation, to 250 Klett units for nitrogenase and hydrogenase measurements. The following relationship between dry weight and Klett units occurs (Daday *et al.*, 1979):

$$100 \text{ Klett units} \equiv 3.3 \text{ mg dry wt./10 ml}$$

Nitrogenase and hydrogenase assays

Nitrogenase was measured by acetylene reduction and hydrogenase as H₂ evolution from dithionite-

reduced Methyl Viologen (Lambert & Smith, 1980). Rates represent averaged measurements over a 1½–2 h incubation, during which time linear progress curves were obtained.

O₂ evolution and uptake

O₂ exchange was measured with a Clark-type oxygen electrode (Rank Bros., Bottisham, Cambridge, U.K.). Suspensions of cells (3 ml) in the electrode compartment were illuminated by a projector (quartz–halogen 24 V/150 W lamp).

Dry-weight determinations

All results are expressed in terms of dry weights of cyanobacteria, determined by heating duplicate samples (10 ml) overnight at 85°C and correcting for the weight of mineral salts in the medium.

CO₂-fixation assays

These were performed by measuring the pH change occurring when cells were incubated in NaHCO₃ (5 mM) in the light (7000 lx) (Laczkó & Barabás, 1981) in closed containers (16 ml of culture in 36 ml Erlenmeyer flasks).

Chemicals and gases

Methyl Viologen was obtained from Sigma Chemical Co., St. Louis, MO, U.S.A. $\text{Na}_2\text{S}_2\text{O}_4$ was purchased from E. Merck A.G., Darmstadt, Germany. Acetaldehyde (laboratory reagent) was obtained from Hopkin and Williams, Chadwell Heath, Essex, U.K. It was kept on ice or at 4°C during solution preparation and injected into assay flasks after gas mixtures were constituted. All experiments with acetaldehyde were done in gas-tight containers. All gases were of the highest purity available from Commonwealth Industrial Gases, Canberra, A.C.T., Australia.

Results

Inhibition of nitrogenase activity

Nitrogenase activity of intact filaments was measured as a function of acetaldehyde concentration (Fig. 1). Because acetaldehyde is known to react with groups such as thiol and amino groups,

which would be at high concentrations in living cells, the degree of inhibition was measured as a function of cyanobacterial concentration to test whether the inhibition curve (Fig. 1) was, in part, a titration curve of total reactive groups. For this experiment an acetaldehyde concentration (10 mM) giving partial inhibition was used. The results (not shown) showed that when the rates were normalized to a constant cell concentration the degree of inhibition was independent of concentration up to 600 Klett units.

The time-dependence of the inhibition by 10 mM-acetaldehyde was also tested. It was shown (Fig. 2) that the inhibition was time-dependent, but was essentially complete within 1 h. In all experiments with 50 mM-acetaldehyde, total inhibition was always observed within a few minutes. In subsequent experiments, including all those reported below, cells were preincubated for 1 h before assay. The acetaldehyde remained during the assays, resulting in linear progress curves.

Fig. 1. *Effect of concentration of acetaldehyde on its inhibition of nitrogenase, hydrogenase and CO_2 fixation*
Cells were treated and assayed as described in the text. Rates refer to initial rates measured over 2 h (for nitrogenase and hydrogenase) or 30 min (for bicarbonate consumption). Algal concentrations were approx. 250 Klett units for nitrogenase and hydrogenase assays and 80 Klett units for the CO_2 -fixation assays. For these CO_2 -fixation assays dark controls were included and gave zero rates. The symbols represent acetylene reduction (●), hydrogenase activity (○) and CO_2 fixation (△).

Fig. 2. Effect of preincubation time on acetaldehyde inhibition of nitrogenase activity

Cells were treated with acetaldehyde (10 mM) (●), and the rate of acetylene reduction was measured after a preincubation time as indicated. Rates for untreated cultures are shown for comparison (○). Cultures were kept in room light (approx. 3000 lx) during the preincubation period.

The reversibility of the inhibition was also tested by centrifuging and washing the cells after acetaldehyde treatment. With 50 mM-acetaldehyde the nitrogenase inhibition was totally irreversible. Interestingly, the washing of the cells resulted in a higher nitrogenase rate in the control cells than existed before washing.

Inhibition of hydrogenase activity by acetaldehyde

Nitrogenase activity, as measured in an intact cell, is a complex process, representing, not only the functioning of the enzyme itself, but also the proper functioning of the ATP- and reductant-generating processes. It was therefore decided to test the effect of acetaldehyde on the closely related enzyme hydrogenase, which is present in *A. cylindrica* cells (Daday *et al.*, 1979; Lambert & Smith, 1981); this enzyme does not require photosynthetically generated products for its activity to be assayed. The inhibition profile, shown in Fig. 1, is very similar to that for nitrogenase. As with nitrogenase, the inhibition could not be reversed by washing the cells with fresh medium (results not shown).

Effect of acetaldehyde on cell growth

Acetaldehyde at a concentration of 50 mM prevented cell growth and soon resulted in cell death.

Effect of acetaldehyde on CO₂ fixation

Having shown that nitrogenase, and hence N₂ fixation, was inhibited by acetaldehyde, we decided to see whether there was any differential effect on CO₂ fixation. The results are also shown in Fig. 1 and reveal that there is no significant or useful differential effect on the two processes.

Effect of acetaldehyde on photosynthetic O₂ evolution and electron transport

Preliminary experiments showed that acetaldehyde (50 mM) was markedly, but not totally,

Fig. 3. Effect of Methyl Viologen on O₂ exchange by *A. cylindrica* and the lack of acetaldehyde inhibition of the Hill reaction

Cells (3 ml) were placed in an oxygen electrode, and the light was turned on at zero time. Methyl Viologen (MV) was injected to a final concentration of 1 mM, NaN₃ to a concentration of 2 mM and acetaldehyde to a concentration of 50 mM, at the indicated times. The rates of O₂ evolution at time 1 and of O₂ uptake at time 2 were 134 and 74 nmol/min per mg respectively. The 3 ml of cells contained 2.19 mg dry wt. The ordinate refers to the total amount of O₂ in the 3 ml volume.

inhibitory towards whole-cell O_2 evolution in the light. To test whether this was due to an effect on light-mediated electron transport, or simply due to an inhibition of the electron-accepting pathways of CO_2 and N_2 fixation (demonstrated above), we tested the effect of acetaldehyde on the Hill reaction, as measured by Methyl Viologen-mediated O_2 uptake (Mehler reaction) in the presence of NaN_3 to inhibit catalase (Trebst, 1972; Izawa, 1980). As shown in Fig. 3, Methyl Viologen (1 mM) produces a net uptake of O_2 in whole cells within a few minutes of its addition, and hence is useful for measurements of the Hill reaction with intact cells. As also shown in Fig. 3, acetaldehyde, at concentrations up to 50 mM, had no effect on this reaction.

In control experiments it was shown that neither Methyl Viologen (1 mM) nor acetaldehyde (50 mM) altered the rate of O_2 uptake in the dark observed with whole cells.

Discussion

The present work resulted from the finding that the O_2 trap described in the introduction resulted in inhibition of nitrogenase activity in *A. cylindrica*. Because of the inhibition of both N_2 fixation and CO_2 fixation, which results from the acetaldehyde formed by this trap, it is clear that caution must be taken in using it with photosynthetic systems. Because of the stoichiometric relationship between O_2 consumed by the trap and acetaldehyde produced, inhibitory concentrations of the latter may easily accumulate during the course of a typical experiment. In fact, there are very few satisfactory O_2 traps that can be used in solution with biological material, and so it is fortunate that the stoichiometry of the glucose oxidase and catalase reactions is such that a net uptake of O_2 still occurs in the absence of ethanol, albeit with a halved efficiency. Alternatively, it may be possible to retain the maximum efficiency of the trap by coupling it with alcohol dehydrogenase and NADH, such that ethanol is regenerated. This would be particularly attractive if suitable NADH-regenerating enzyme systems were available.

Comment is required on the use of Methyl Viologen to measure the Hill reaction in intact cells (Fig. 3). Conflicting statements appear in the literature concerning the permeability of biological

membranes to this substance. It is said to be impermeant in *Thiocapsa roseopersicina* (Bagyinka *et al.*, 1982) and *Escherichia coli* (Jones & Garland, 1977), but there is increasing evidence of its permeance in cyanobacterial cells (Nikitina & Gusev, 1979; Ochiai *et al.*, 1980; Smith *et al.*, 1983), including *A. cylindrica* (Tözüm & Gallon, 1979).

Although acetaldehyde is a molecule that is likely to be reactive with a great number of side groups found in biological tissues and enzymes (it forms acetals with alcohol, thiol and amino groups, for example), the fact that it does not affect electron flow through the photosystems, as measured by the Hill reaction with Methyl Viologen-mediated O_2 uptake, is of considerable interest. This observation is of potential use for allowing, by means of a rapidly acting water-soluble inhibitor, photosynthetic electron flow to be measured without N_2 and CO_2 fixation.

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