Hydrogen Evolution by Nitrogen-Fixing Anabaena cylindrica Cultures

Abstract. *Actively growing, nitrogen-fixing cultures of the blue-green alga* Anabaena cylindrica *can simultaneously evolve hydrogen and oxygen from water and light. Hydrogen evolution was strongly inhibited by* N_2 *but only slightly by CO or* O_2*, characteristics of the nitrogenase reaction in the heterocysts of* Anabaena cylindrica. *We suggest that this reaction has potential use in solar energy conversion.*

Hydrogen evolution from water and light with a chloroplast-ferredoxin-hydrogenase system has been demonstrated (*1*) and photosynthetic hydrogen production has been suggested as a method of solar energy conversion. The oxygen generated from water by the photosynthetic apparatus inhibited the hydrogen evolution reaction, limiting the efficiency of the system (*1*). Hydrogen evolution by either green algae (*2*) or photosynthetic bacteria (*3*) is also very oxygen labile and does not occur together with oxygen evolution (*4*). We have now found that *Anabaena cylindrica*—a heterocystous, filamentous, blue-green alga—can photochemically evolve hydrogen and oxygen from water.

All experiments were carried out with axenic *Anabaena cylindrica* (strain B629) cultures actively growing under fluorescent lamps (General Electric F400W cold white; the light intensity at the side of the vessels was 2×10^4 erg cm^{-2} sec^{-1}) in an atmosphere of air and CO_2 (99.7 : 0.3, by volume) under nitrogen-fixing conditions (*5*). The cultures evolved hydrogen in an atmosphere of argon or argon and O_2 (82 : 18, by volume) (Table 1). In the presence of nitrogen, hydrogen evolution was inhibited by more than 85 percent; however, 2 percent CO restored hydrogen evolution almost to the original value (Table 1). Inhibition by nitrogen and insensitivity to CO are characteristics of the hydrogen evolution reaction catalyzed by the nitrogenase (*6*). Hydrogen evolution is a side reaction of the nitrogenase-catalyzed reduction of atmospheric nitrogen to ammonia. Nitrogenase activity is routinely measured by the ability of the enzyme to reduce acetylene to ethylene [see (*7*) for reviews].

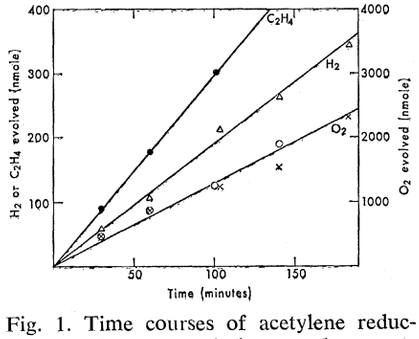

Fig. 1. Time courses of acetylene reduction, hydrogen evolution, and oxygen evolution by *Anabaena cylindrica* under argon. Oxygen evolution was determined by means of a three-way argon flushed valve (to avoid air contamination) according to the procedure used for the hydrogen assays. The culture had a cell density of 0.10 absorbancy unit. ●, Acetylene reduction; △, hydrogen evolution; ×, oxygen evolution during hydrogen evolution; and ○, oxygen evolution during acetylene reduction.

It is believed that in *Anabaena cylindrica* nitrogenase is localized in the heterocysts (*8*), distinctly differentiated cells occurring at intervals among the vegetative cells of the filament. In the vegetative cells light-driven oxygen evolution fom water coupled to CO_2 fixation takes place. Some of the CO_2 fixed in vegetative cells is transferred to the heterocysts (*9*) where it is used in the nitrogen fixation reactions. This model (*5*) accounts for the common observation that the photosynthesis (oxygen evolution) inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) usually has little immediate inhibitory effect on acetylene reduction (Table 1), an indication that there was no direct coupling between oxygen evolution and nitrogenase (*10*). The oxygen-sensitive nitrogenase is protected against oxygen inactivation by some unknown mechanism operating in the heterocysts, as evidenced by the slight inhibitory effect of atmospheric oxygen on acetylene reduction (Table 1). Since the inhibition of hydrogen evolution by O_2 or DCMU was less than 30 percent (Table 1), this supports the view that the nitrogenase activity in the heterocysts is responsible for the observed hydrogen evolution.

Light was required for both hydrogen evolution and acetylene reduction under argon (activities more than 95 percent inhibited in the dark). However, in the presence of O_2, acetylene reduction in the dark was almost 50 percent of that in the light (Table 1). Although hydrogen evolution and acetylene reduction are catalyzed by nitrogenase in vitro at the same rate, the algal cultures showed higher acetylene reduction rates than hydrogen evolution rates under all conditions (Table 1). This can be explained by the presence of an uptake hydrogenase that competes with the nitrogenase-mediated hydrogen evolution reaction. Such a hydrogen uptake reaction can be demonstrated by the fact that after a 24-hour incubation under argon in the light in the presence of DCMU ($2 \times 10^{-5} M$) acetylene reduction under argon decreased by 90 percent, whereas it was normal under hydrogen. A twofold stimulation of acetylene reduction by hydrogen has been reported for air-grown cultures (*11*); however, our untreated cultures grown on air/CO_2 exhibited no such stimulation. When cell densities of the cultures increased and growth rates decreased (*12*), the hydrogen uptake reaction increased and net hydrogen evolution was only 10 percent of acetylene reduction.

Time courses of hydrogen evolution and acetylene reduction under argon and light were linear over 3 hours. The rates of O_2 evolution observed during both sets of nitrogenase assays were similar to each other (Fig. 1). Oxygen

Table 1. Hydrogen evolution and acetylene reduction by *Anabaena cylindrica* cultures grown in air and CO_2 (99.7 : 0.3, by volume). Results are given as nanomoles of product per minute per milliliter. *Anabaena cylindrica* B-629 was grown in pure culture as described (*5*). Cells (2 ml) were injected into Fernbach flasks (7.5 ml) containing all gases and additions indicated (including 10.5 percent acetylene in the acetylene reduction assays). The flasks were immediately vented and incubated with shaking at 30°C at a light intensity of 6×10^4 erg cm^{-2} sec^{-1} (General Electric 30W reflector lights). After 45 minutes, 0.25 ml of 20 percent trichloroacetic acid was injected to terminate the reaction. Ethylene and hydrogen were determined as described (*1, 5*), except that a Varian Aerograph 1400 and a Hewlett-Packard 5700A gas chromatograph were used, respectively. The culture had a cell density of 0.09 absorbancy unit (Klett filter, 660 nm) [0.002 absorbancy unit corresponds to 3.4 μg (dry weight per milliliter)].

Assay conditions	Hydrogen evolution	Acetylene reduction
Argon, light	1.24	1.52
N_2, light	0.16	1.36
N_2, + 2% CO, light	1.05	
Ar + $2 \times 10^{-5} M$ DCMU, light	0.96	1.32
Ar + 18% O_2, light	0.87	1.22
Ar + 18% O_2, dark	0.20	0.56

evolution was severalfold higher than acetylene reduction because CO_2 fixation in these growing cultures was a metabolically more active reaction than nitrogen fixation. If the cultures were starved for nitrogen by flushing for 40 hours with a mixture of argon and CO_2 (99.7 : 0.3) or a mixture of argon, O_2, and CO_2 (79.7 : 20 : 0.3) in the light, oxygen evolution was sharply reduced (more than 90 percent), while hydrogen evolution, heterocyst frequency, and [as reported (*13*)] acetylene reduction increased. In these nitrogen-starved cultures, hydrogen evolution in the dark was severalfold higher than acetylene reduction in the dark, thus some of the hydrogen evolution appeared to be due to the reported reversible hydrogenase activity of *Anabaena cylindrica* (*14*).

Our data demonstrate that the heterocyst-vegetative cell system can be used to simultaneously produce hydrogen and oxygen from water and light energy. Although normally more oxygen than hydrogen is produced, manipulation of the cultures (as by nitrogen starvation) should allow the achievement of stoichiometric ratios. Although about a 10 percent conversion of solar energy into chemical energy can be theoretically achieved with photosynthetic processes, such an efficiency cannot yet be achieved or sustained in hydrogen production by heterocystous blue-green algae. However, we have shown the basic requirement for solar energy conversion: the decomposition of water into hydrogen and oxygen by light energy.

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Norepinephrine-Sensitive Adenylate Cyclases in Rat Brain: Relation to Behavior and Tyrosine Hydroxylase

Abstract. *Responses of norepinephrine-sensitive adenosine 3′,5′-monophosphate (cyclic AMP)–generating systems in combined midbrain-striatal slices of four rat strains correlate positively with spontaneous behavioral activity and negatively with levels of midbrain and striatal tyrosine hydroxylase. Responses of cerebral cortical norepinephrine-sensitive cyclic AMP systems correlate negatively with spontaneous behavioral activity and positively with midbrain and striatal tyrosine hydroxylase. Such correlations were not found with responses of the cyclic AMP–generating systems to isoproterenol, adenosine, veratridine, or of an adenosine and norepinephrine combination.*

The proposal of an interrelation between brain biogenic amines and behavior (*1*) and its subsequent elaboration (*2*) have been followed by numerous attempts to demonstrate a causal relation between steady state levels of brain biogenic amines and spontaneous behavioral activity in various rat and mouse strains. The results obtained from such studies (*3*) have been contradictory and unclear and emphasize our lack of understanding of the neurochemical correlates of behavior. Recently, however, an excellent negative correlation between spontaneous behaviorial activity and levels of tyrosine hydroxylase in midbrain ($r = -.94$) and striatum ($r = -.83$) was reported for several rat strains (*4*). It would, therefore, appear that the levels of tyrosine hydroxylase, the rate-limiting step in catecholamine biosynthesis (*5*), may provide a more appropriate measure of a functional pool of newly synthesized catecholamine, which is preferentially released during adrenergic neuronal activity (*6*) than does the steady state levels of catecholamines. The negative correlation between spontaneous motor activity and tyrosine hydroxylase activity was proposed (*4*) to indicate that low levels of transmitter biosynthesis are associated with a relatively high degree of functional activity of adrenergic receptor-mediated mechanisms and that this enhanced activity results in a high level of spontaneous behavioral activity. Conversely, high levels of transmitter biosynthesis were proposed to be associated with a relatively low degree of functional activity of adrenergic receptor-mediated mechanisms with a resultant low level of spontaneous behavioral activity. The functional activity of adrenergic receptor-mediated mechanisms could depend, of course, on at least two factors: (i) the number of activations of adrenergic receptors by "released" neurotransmitter norepinephrine, and (ii) the magnitude of the resultant biochemical signal from each activation.

Formation of adenosine 3′,5′-monophosphate (cyclic AMP) has been shown to be intimately associated with neuronal transmission in both the central (*7*) and peripheral (*8*) nervous systems. Accumulations of cyclic AMP have been demonstrated in brain tissue after stimulation by electrical means (*9*), depolarizing agents (*10*), adenosine (*11*), and biogenic amines (*12*). The proposed relation between tyrosine hydroxylase activity, receptor function, and spontaneous motor activity (*4*) and the possibility that an adenylate cyclase system is associated with adrenergic receptors if it is not the receptor itself (*13*) led us to investigate a possible association between the magnitudes of spontaneous motor and tyrosine hydroxylase activity and the norepinephrine-elicited accumulation of cyclic AMP in cerebral cortex and midbrain and striatum of various rat strains.

Adult male rat strains F344, ACI, and BUF were obtained from Microbiological Associates, Inc., Walkersville, Maryland, and adult male Sprague-Dawley rats were obtained from Taconic Farms, Germantown, New York. The rats were housed at our facilities for at least 1 week before use. Animals (175 to 225 g) were killed by decapitation, and cortical