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Complementary Functioning of Nitrogenase Components from a Blue-Green Alga and a Photosynthetic Bacterium

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Nitrogenases from *Anabaena cylindrica* and *Chloropseudomonas ethylicum* were partially purified into two components. *A. cylindrica* fraction I protein complemented fraction II protein from *C. ethylicum*. However, the reciprocal cross between *C. ethylicum* fraction I and *A. cylindrica* fraction II was negative.

We recently demonstrated nitrogenase activity in cell-free extracts of the blue-green alga *Anabaena cylindrica* (7). The substrate requirements for nitrogenase activity in these preparations were the same as those reported for bacterial nitrogenase (5). Nitrogenase has been purified from a number of species of bacteria and it has been shown that in all preparations two protein components (fraction I and fraction II) are required for nitrogenase activity. The two component proteins from different organisms appear to be very similar, and in many cases enzymatic activity is obtained when fraction I and fraction II from different organisms are combined (1, 2, 6).

We have now partially purified the nitrogenase from *A. cylindrica* by chromatography on diethylaminoethyl (DEAE) cellulose and demonstrated that it can be separated into two components. We have also investigated the complementary activity of the blue-green algal nitrogenase components with components prepared from the green photosynthetic bacterium *Chloropseudomonas ethylicum*.

Extracts were prepared from *A. cylindrica* by the procedure described previously (7), except that 0.01 M MgCl was added to the buffer. The crude extract was then centrifuged for 1 hr at 78,000 × *g*. Mucilagenous material present in the extracts described previously was sedimented in the presence of 0.01 M $MgCl_2$. The *C. ethylicum* nitrogenase extract was prepared as described by Evans and Smith (4). Nitrogenase fractions I and II were prepared from the *A. cylindrica* and *C. ethylicum* extracts by DEAE chromatography essentially as described by Detroy et al. (2). Fraction I was eluted from the DEAE column with 0.28 M NaCl in 0.02 M tris-(hydroxymethyl)aminomethane (Tris)-chloride (*p*H 7.5) containing 0.0025 M $Na_2S_2O_4$. Fraction II was eluted with 0.6 M NaCl in 0.02 M Tris-chloride (*p*H 7.5) containing 0.0025 M $Na_2S_2O_4$.

When nitrogenase activity was assayed as described previously (7) by measuring the reduction of acetylene to ethylene (3), the individual components were essentially inactive (Table 1). The combination of fractions I and II was re-

TABLE 1. *Acetylene reducing activity and cross reactions of nitrogenase fractions I and II from Anabaena cylindrica and Chloropseudomonas ethylicum*[a]

Fractions		C_2H_4 formed (nmoles)
A. cylindrica	*C. ethylicum*	
I		18
II		9
I + II		247
I	II	320
	I	4
	II	9
II	I	13
	I + II	495

[a] Reaction mixture contained, in a final volume of 1.5 ml: potassium HEPES (*p*H 7.5), 100 μmoles; $MgCl_2$, 10 μmoles; adenosine triphosphate, 4 μmoles; creatine phosphate, 10 μmoles; creatine kinase, 50 μg; sodium dithionite, 5 μmoles. The concentrations of protein used in the assay procedure were as follows: *A. cylindrica* I, 2.1 mg; *A. cylindrica* II, 1.1 mg; *C. ethylicum* I, 1.2 mg; *C. ethylicum* II, 1.5 mg. The gas phase was 90% Argon-10% acetylene. The reaction was carried out in 7.0-ml glass vials sealed with Subaseal rubber stoppers. After incubation for 10 min at 30 C, the reaction was stopped by injecting 0.1 ml of 60% $HClO_4$.

quired for activity. When fraction I from *A. cylindrica* was combined with fraction II from *C. ethylicum*, the rate of acetylene reduction was similar to that obtained when fractions I and II were combined from *A. cylindrica*. However, when fraction I from *C. ethylicum* was combined with fraction II from *A. cylindrica*, essentially no activity was obtained.

These results show that, like nitrogenase from other organisms, the nitrogenase from photosynthetic bacteria and blue-green algae consists of two components. In general, the ability of the two components from different organisms to complement each other is not completely predictable (1, 2, 6). However, because complementation does occur in many cases, this may be evidence for the basic similarity of nitrogenase from different organisms. Because fraction I from *A. cylindrica* complements fraction II from *C. ethylicum*, this suggests that, despite the many differences between the blue-green algae and the bacteria, the nitrogenases are similar and the mechanism of nitrogen reduction in blue-gree algae is the same as in bacteria.

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