

Nitrogen Fixation and Photosynthesis in a Unicellular Blue-Green Alga

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Newer Mass Spectrometric and Chromatographic Techniques in Taxonomic Studies of Erythrina Alkaloids

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Gloeocapsa sp. is the only unicellular blue-green alga shown so far to fix atmospheric dinitrogen, as measured by the acetylene reduction technique [1]. During the growth of cultures of *Gloeocapsa*, photosynthesis is maintained at a low level of activity over the period of maximum nitrogen fixation, which may be important in protecting the oxygen-sensitive nitrogenase from inhibition by photosynthetically produced oxygen [2].

Photosynthesis may nevertheless be expected to provide the ATP and reducing power necessary for the reduction of acetylene by *Gloeocapsa* nitrogenase in the light, but since the alga can reduce acetylene in the dark at significant rates for up to 4 hr, dark-generated reductant and ATP are also available to the enzyme. *In vitro* studies suggest that reductant may be supplied in the dark from NADPH, reduced by isocitrate dehydrogenase and, to a lesser extent, malate dehydrogenase [3].

Fluoroacetate, by its conversion to fluorocitrate, inhibits aconitate hydratase and thereby prevents the formation of isocitrate from citrate. Complete inhibition of *Gloeocapsa* nitrogenase was observed in whole cells incubated in the light with 10 mM fluoroacetate for 3-4 hr. If, as seems likely, this inhibitor prevents the regeneration of substrates (isocitrate and probably malate also) which can provide reducing power for nitrogenase, then it appears that these substrates rather than photosynthesis are supplying this reductant, even in the light. This rather surprising observation may be explained if there is a physical separation of nitrogenase and photosynthesis within the single cell of *Gloeocapsa*, which while possibly contributing to the protection of nitrogenase from oxygen inactivation, does so only at the expense of isolating the enzyme from photosynthetically produced reductant.

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The use of GC-MS has enabled us to rapidly screen some 20 species of *Erythrina* (Leguminosae) seeds for their alkaloid content [1]. Alkaloids were usually present both in the free state, and as glycosides, and the latter were hydrolysed before extraction; free hydroxyl groups were derivatized as their trimethyl silyl ethers prior to GC-MS. Field ionization and field desorption mass spectrometry have now also proved to be very useful for the direct analysis of mixtures of underivatized alkaloids (especially the less volatile phenolic materials) as under optimum conditions only the molecular ion is formed and no fragmentation occurs. We have already described [2] some results with field ionization GC-MS, and we have now shown the great potential of high pressure liquid chromatography combined with field desorption mass spectrometry for the rapid analysis of underivatized and involatile alkaloids.

These preliminary experiments have shown the presence of large numbers of new alkaloids, many of which are oxygenated in the 11-position of the *Erythrina* alkaloid skeleton. The 11-oxygen function appears to be the most significant taxonomic difference between *Erythrina* species in the Eastern hemisphere (which we have studied) and those occurring on the American continent (studied by Rinehart *et al.* [1]).

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