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CHAPTER II

SULFUR CYCLING AND METABOLISM OF PHOTOTROPHIC AND FILAMENTOUS SULFUR BACTERIA

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Introduction

Sulfur, an abundant element in the biosphere, is rarely a limiting nutrient for organisms. Its proportion in living material has been estimated to be 1 atom of S for 15 atoms of N and 100 atoms of C. The element sulfur exists in oxidation states from -2 to +6 in organic and inorganic molecules. Microorganisms catalyse the oxidation and reduction of different forms of sulfur, establishing a cycle that involves sulfur incorporation into organic matter (anabolic, structural, slow cycling) or the use of different sulfur compounds as acceptors or donors of electrons (catabolic, energetic, rapid cycle). Nonassimilatory sulfur metabolism coupled with the carbon cycle may represent the oldest energy cycle in the biosphere, one used by the earliest autotrophic prokaryotes to obtain energy (Clark, 1981).

Hydrogen sulfide is a highly reactive, extremely toxic compound subject to both biological and nonbiological oxidation. Sulfide can be oxidized to sulfur and sulfate by bacteria under aerobic as well as anaerobic conditions. Some bacteria oxidize sulfide aerobically to generate energy. *Beggiatoa* and *Thiothrix*, for instance, are filamentous, microaerophilic bacteria capable of oxidizing sulfide, and depositing sulfur globules within the cells:

$$2\ H_2S + O_2 \longrightarrow 2S + 2\ H_2O$$

In the absence of sulfide, the sulfur globules are oxidized further to sulfate. These are typical "gradient organisms," positioning themselves in the interfaces of anaerobic environments (sulfate-reducing sediment or sulfide-rich layers of water) with overlying, partially oxygenated waters.

Hydrogen sulfide is also subject to biological photooxidation in anaerobic environments. Phototrophic sulfur bacteria (Chromatiaceae and Chlorobiaceae) are able to photoreduce carbon dioxide while oxidizing sulfide, first to elemental sulfur and later to sulfate (CH_2O symbolizes photosynthate):

$$CO_2 + 2\ H_2S \longrightarrow CH_2O + 2S + H_2O$$
$$3\ CO_2 + 2S + 5\ H_2O \longrightarrow 3\ CH_2O + 2\ H_2SO_4$$

Chromatiaceae are also capable of accumulating internal sulfur globules (the genus *Ectothiorhodospira* is the only exception, and accordingly will be separated into a new family). Some Chromatiaceae can tolerate low concentrations of oxygen and thus are also considered "gradient organisms."

Although oxidation of sulfide to sulfate by different microorganisms is well known, the use of internal sulfur globules as electron acceptors to oxidize or derive energy from storage compounds such as glycogen in the absence of an external source of energy (endogenous metabolism) was hypothesized a long time ago (Dawes and Ribbons, 1964; van Gemerden, 1968) but not definitively demonstrated.

To investigate different aspects of the ecophysiology of purple and green bacteria the following studies were performed:

1. Phototrophic sulfur bacteria taken from different habitats (Alum Rock State Park, Palo Alto salt marsh, and Big Soda Lake) were grown on selective media, characterized by morphological and pigment analysis, and compared with bacteria maintained in pure culture.

2. A study was made of the anaerobic reduction of intracellular sulfur globules by a phototrophic sulfur bacterium (*Chromatium vinosum*) and a filamentous aerobic sulfur bacterium (*Beggiatoa alba*).

3. Buoyant densities of different bacteria were measured in Percoll gradients. This method was also used to separate different chlorobia in mixed cultures and to assess the relative homogeneity of cultures taken directly or enriched from natural samples (including the purple bacterial layer found at a depth of 20 meters at Big Soda Lake.)

4. Interactions between sulfide-oxidizing bacteria were studied. Pairs of sulfide-oxidizing species competed for electrons (sulfide was the only available electron donor in the medium common to a purple sulfur bacterium (*Chromatium vinosum*), a green sulfur bacterium (*Chlorobium phaeobacteroides*) and a cyanobacterium (*Oscillatoria limnetica*)). These bacteria, selected because of their sulfide requirements and the fact that they can co-exist in aquatic enviroments where intense gradients occur, were handled pairwise by placement in a common medium separated by a membrane filter. Competition between two of these species at a time was measured under conditions where metabolites and toxins (but not cells) passed easily through the common culture medium.

References

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