

N92-13626

THE EFFECTS OF OXYGEN ON THE EVOLUTION OF MICROBIAL MEMBRANES

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Extant microorganisms are an invaluable resource for furthering our understanding of Earth's evolution. Perhaps the greatest influence on this process has been the rise in levels of free oxygen. While the roots of biochemical pathways are anaerobic, oxygenase-type mechanisms did evolve once sufficient molecular oxygen was available. The products of these oxygen-requiring syntheses are more characteristic of eukaryotic organisms and must have been particularly important for the development of these more highly evolved cells. In a number of cases, examples of this evolutionary transition exist in extant prokaryotes and are of importance to furthering our understanding of cellular evolution. This is particularly true for the biosynthesis of sterols and hopanoids. With few exceptions, sterols are a ubiquitous constituent of eukaryotic membranes, however, only a few prokaryotes are known to synthesize them. Hopanoids, on the other hand, are present in many bacteria. Both compounds are cyclic triterpenes, products of the cyclization of squalene. While hopanoid synthesis is anaerobic, sterol synthesis involves several oxygen-requiring enzymes. Ourisson has suggested that hopane polyols are primitive analogues of the sterol molecule acting as membrane stabilizers, and, indeed, model membrane studies have suggested that hopanoids may perform a cholesterol-like function in bacterial membranes. One prokaryote, Methylococcus capsulatus, synthesizes both hopanoids and sterols and, thus, provides a unique opportunity to study the evolution of membrane function.

Methane-oxidizing bacteria, such as M. capsulatus, are characterized by the presence of three potentially distinct membrane systems: an outer, cell boundary layer; an inner or pericytoplasmic layer; and an extensive intracytoplasmic membrane system. When the outer and cytoplasmic membranes of this organism were separated by sucrose density centrifugation, lipid analysis showed that the sterol and hopane polyol (the principal hopanoid in this organism) were associated predominantly with the outer membrane. The molar ratio of phospholipid to hopane or sterol in the cytoplasmic membrane was approximately 30:1, similar to the ratio for whole cells. However, the ratios for outer membrane were 11:1 for hopane and 6:1 for sterol. When M. capsulatus was grown at different temperatures, lipid analysis of the whole cells showed that both sterol and unsaturated fatty acid levels decreased at higher growth temperatures; sterol concentrations were 0.116 $\mu\text{mole}/\mu\text{mole}$ phospholipid at 30°C and 0.025 $\mu\text{mole}/\mu\text{mole}$ phospholipid at 45°C, while the saturated to unsaturated fatty acid ratio increased from 0.397 to 1.475. Hopane polyol levels were constant over this temperature range, however, methylation of the A-ring (a characteristic modification of the hopane molecule in some methane-oxidizers) decreased markedly in cells grown at 30°C. These results imply that sterol and hopane molecules are required for enhancement of some specific membrane function, potentially by modulating membrane fluidity.