BIOSURFACTANTS

Production • Properties • Applications

edited by
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BIOSURFACTANTS

Preface

Following our first book on biosurfactants (*Biosurfactants and Biotechnology*, Marcel Dekker, 1987), this book further expands this exciting and relatively new field of biotechnology. In the last five years, interest in and active research on, as well as applications of, biosurfactants, have increased. Although initial interest and applications were primarily in the area of petroleum engineering and enhanced oil recovery, new applications in medicine and industry have evolved. Because of their advantages over synthetic surfactants, biosurfactants are also of increasing interest in cosmetics, foods, environmental control and abatement, and in any industry where surface-active phenomena play a role in processing and product formulation.

The 17 chapters in this book have been written by leading international authorities on these topics, representing the state of the art. Further potential applications of biosurfactants are shown in a variety of processes and products. In this respect, this book is an excellent resource for both research and development and industrial use.

Biosurfactants have also found wide and excellent application in environmental management, as they have been demonstrated to help and enhance biodegradation of toxic pollutants in water and soil. At the present time not much information is available in the literature, but there is a solid indication that many private companies are actively pursuing research in this direction, as well as in applications of biosurfactants in cosmetics and foods. One can predict with confidence that the application of biosurfactants in these areas will be further enhanced in the future.

At this stage, biosurfactants as high-value biotechnological products have been fully recognized worldwide. It took about 25 years to reach this state, after the

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Production

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Biosynthetic Mechanisms of Low Molecular Weight Surfactants and Their Precursor Molecules

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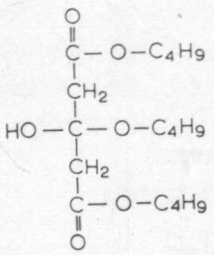
I. INTRODUCTION

Biosurfactants display a range of structures but have the common ability to cause emulsification of oil–water mixtures. Accordingly, biosurfactants must be able to dissolve, at least partially, in both water and a water-immiscible liquid, thereby effecting a decreased surface tension enabling mixing and microsolubilization (i.e., emulsification) to occur. The range of biological components that are available as building blocks for any biosurfactant is limited. They may vary from components that are wholly water-soluble but have no solubility in oil to those that are virtually water-insoluble but can, and do, dissolve in any oil or lipid material.

Biological molecules of the first category include the carbohydrates, especially the mono- and disaccharides. Also in this category are the polyols that are derived from the carbohydrates, as well as a number of the hydrophilic amino acids, for example, glutamate, aspartate, lysine, ornithine, and arginine. One should also include the principal nucleotide bases—guanine, cytosine, adenine, and thymine—but, although these are water-soluble, they do not appear to be used in any of the biosurfactants whose structure has been established. Peptides consisting primarily of hydrophilic amino acids are also water soluble and the acids associated with the tricarboxylic acid cycle are strongly water-soluble biological molecules. The tricarboxylic acid, citric acid, is of course produced from *Aspergillus niger* on a commercial scale and processes for the production of the dicarboxylic acids, fumaric acid, and malic acid also exist but have limited commercial productions [1]. Biosurfactants based on them or related acids have yet to be reported through chemically produced compounds, such as tributylcitrate [1] are clearly useful in the surfactant industry.



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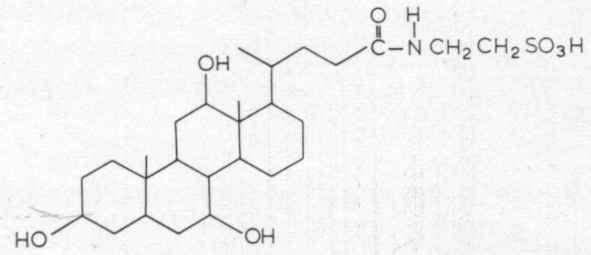

[2]

For water-insoluble biomolecules, the range naturally includes all of the microbial lipids whose molecular complexities go from those based on fatty acids to those based on isoprenoid structures, for example, sterols, terpenes, carotenes, and polyprenols [2]. Although terpenoid compounds are not known to be involved in any of the molecules usually considered as biosurfactants, it should be pointed out that taurocholic acid [2] which is derived from the triterpene, cholesterol, and the amine, taurine is the lipoidal emulsificant used in mammalian lipid metabolism to effect the dispersion of fatty materials in aqueous environments. Such biosurfactants, although adequately produced in animals as a bile acid, do not appear to have any microbial counterpart that has so far been identified as a lipid-emulsifying agent. However, also included here are the hydrophobic amino acids, such as phenylalanine, leucine, isoleucine, valine, and alanine, which have only a very limited solubility in water. Polypeptides that have a predominance of such residues also have limited solubilities in water with a concomitant increase in their ability to associate with lipids especially at lipid–water interfaces, where biosurfactants exert their greater influence.

A biosurfactant is usually found to be a combination of water-soluble and water-insoluble components, thus enabling it to associate at any water–oil interface so that mutual solubilization or emulsification may begin. Although at first inspection one may be surprised at the apparently large range of biosurfactants, the number in fact is somewhat limited as the range of water-soluble to water-insoluble components found in nature is far greater than those actually used by microorganisms. Perhaps as progress continues to be made, we may see new biosurfactants emerging that do incorporate some of the molecules mentioned above that have not yet been implicated as components of microbial surfactants.

In this chapter, we give the pathways of biosynthesis for the principal components of microbial surfactants: fatty acids and related components including the various long-chain acylated components, the carbohydrates and polyol moieties of the glycolipids, and the amino acids used in a number of different ways both as water-soluble entities and as water-insoluble moieties. Greater detail concerning the range of microbial lipids, which are constituents of most biosurfactants, are found in the book on this subject edited by Ratledge and Wilkinson [2], although briefer accounts by Weete [3], Harwood and Russell [4], and Gurr and Harwood [5] may be equally useful.

II. BIOSYNTHESIS OF FATTY ACIDS FROM GLUCOSE

Microbial fatty acids are usually of a narrow range of chain lengths, C_{16} and C_{18}, with relatively small amounts of shorter (C_{12} and C_{14}) and longer (C_{20}) acids. Although the biosynthesis of fatty acids is similar in all biological systems, there are important differences between some bacterial systems and those of eukaryotic

microorganisms (yeasts and molds). It is this difference that then accounts for the difference in how unsaturated fatty acids are synthesized in the two systems and why the resulting unsaturated C_{18} fatty acids are not the same. Bacteria usually produce 18:1 (*c*11),* *cis*-vaccenic acid, whereas yeasts and molds and all other living cells, produce oleic acid, 18:1 (*c*9). These differences occur because of the organization of the enzymes making up the individual fatty acid synthetase complexes. In all cases, however, fatty acid biosynthesis begins with acetyl-coenzyme A (acetyl-CoA). As this is the key intermediate for fatty acid biosynthesis and also, via formation of mevalonic acid, for the biosynthesis of all the terpenoid lipids, it is important to consider the metabolic origins of this molecule.

A. Formation of Acetyl-CoA

In bacteria, there is no mitochondrion and consequently acetyl-CoA is formed directly in the cell compartment (the cytoplasm) from pyruvate (the end-product of glucose metabolism) by pyruvate dehydrogenase:

$$\text{Pyruvate} + NAD^+ + \text{CoA} \rightarrow \text{acetyl-CoA} + CO_2 + NADH \quad (1)$$

In eukaryotic microorganisms, that is yeasts and molds, pyruvate dehydrogenase is a mitochondrial enzyme and thus, although pyruvate can enter the mitochondrion, its product, acetyl-CoA, cannot leave because its molecular size is too large. As fatty acid biosynthesis takes place in the cytoplasm (as in bacteria) there has to be some mechanism for acetyl units to be translocated from inside the mitochondrian into the main cell compartment.

Two principal routes for translocation of acetyl units occur in yeasts and molds:

1. The carnitine acetyl transferase (CAT) route. This system appears to occur in all yeasts and molds. No exceptions have yet been reported. CAT catalyzes the reversible formation of acetyl-carnitine:

$$\text{Acetyl-CoA} + \text{carnitine} \rightarrow \text{acetyl carnitine} + \text{CoA} \quad (2)$$

Acetyl-carnitine, being smaller than acetyl-CoA, is readily transported across the mitochondrial membrane. The reverse of reaction 2 then occurs in the cytoplasm to regenerate acetyl-CoA.

* The standard nomenclature for lipids is used throughout this chapter. Thus, for example, 18:1 (*c*11) refers to a fatty acid having 18 carbon atoms and one double bond; the position of the double bond is then denoted by enumerating the first C atom, starting at the carboxyl end, of the bond and, where needed, whether this is *cis* (*c*) or *trans* (*t*). Thus 18:1 (*c*11) refers to *cis*-vaccenic acid. Branched-chain fatty acids are designated by br, although if the branching is at the ω-1 or ω-2 C atoms these acids are referred to as *iso* and *anteiso* acids, respectively. Cyclopropane or cyclopropene rings are designated by *cyc*. [A fuller discussion and description of microbial fatty acids may be found in Refs. 2–4.]