

MICROBIAL CHEMISTRY

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Summary

Microbial chemistry deals with all the aspects of metabolic events occurring in

microorganisms. This field of science has developed at a tremendous pace, from a mere diagnostic section of bacteriology (*see also – Medical Microbiology*) to an independent branch that links the fields of microbiology, biochemistry and genetics.

One of the most impressive achievements of science has been the explosion of knowledge about the chemical composition of organisms and the way these chemicals interact to create the phenomenon we recognize as life. The special nature of living cells does not reside, however, in unique chemical principles, but rather in the immensely sophisticated way in which they utilize the ordinary laws of thermodynamics and chemistry.

1. Introduction

When considering all life forms occurring in the biodiversity of nature there is no doubt that microorganisms are the most powerful creatures in existence. They determine life and death on this planet. They can mercilessly kill humans, animals and plants, but at the same time they can be harnessed to sustain life. Nature has provided us with a perfect balance in Carbon, Nitrogen and Phosphorous cycles to sustain microbial, plant, animal and human life. Even small interference in these cycles can swing the pendulum very quickly into the direction of killing or sustaining mankind. It is the microorganism, which ultimately determines the growth and existence of plants, animals and humans on this planet. It is therefore of utmost importance that we give microbiology a first priority, as we have to isolate and investigate the biochemistry and behaviour of the microorganism in order to understand how nature works. Only when we obtain this information will we be able to sustain and improve life in our community. The microorganism is much more flexible and adaptable to environmental changes than plants, animals and humans.

2. General Considerations

In many instances, organisms are compared to chemical factories, emphasizing the chemical nature of life and the fact that growth, development and reproduction all depend on chemical reactions. Where Chemical factories and organisms deviate from each other are: chemical factories have been designed to convert specific raw materials into just a few products, whereas evolution has endorsed organisms with the ability to take in a wide range of raw materials or nutrients and transform them into literally thousands of different types of products, each with a specific biological role. Biotechnology draws its strength from these powerful chemical reactions. All of these individual chemical reactions must be harmoniously coordinated and proteins, called enzymes, as well as nucleic acids play a central role at every stage.

Cellular organisms share a common chemical composition. Their most distinctive chemical attribute is manifested in the presence of three classes of complex macromolecules: protein, ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). The latter is the constituent that carries in coded form all the genetic information necessary to determine the specific properties of the organism, known collectively as its phenotype. In all cellular organisms the genetic information is stored as a linear series of bases in DNA. This DNA (of cells) is a double-stranded helix in which the two

strands wind around each other, forming one complete turn approximately every 10 base pairs. The two strands are held together by hydrogen bonds. This basic genomic structure is common to all cells, however, there are differences among the major groups with respect to their chromosomes.

The genetic information is initially transcribed into complementary RNA sequences, in the form of molecules of RNA known as messenger RNA (mRNA), which serve as templates for the synthesis of all the specific protein molecules characteristic of the cell. The translation of the transcribed genetic message is mediated by organelles known as ribosomes, composed of protein subunits and a special class of RNA, ribosomal RNA (rRNA). A third class of RNA molecule, the transfer RNAs also participate in protein synthesis, as carriers of the amino acids that are assembled into linear sequence in the primary step of protein synthesis. The proteins include the enzymes that catalyze the activities, and the subunits from which many classes of proteinaceous cellular microstructures are assembled (*see – Methods in Gene Engineering*).

Protein molecules consist mostly of carbon, oxygen, hydrogen and nitrogen. Every protein is constructed from twenty different sorts of simple building blocks referred to as amino acids. The staggering versatility of proteins arises from the fact that so many different shapes can be created by arranging amino acids in various ways. Once the cell has assembled a chain of amino acids, the chain twists and turns to create a complex three-dimensional structure. The final shape of the protein molecule is determined by the order in which its amino acids are strung together. Some of the proteins are enzymes. These enzymes are biological catalysts, which speed up the rate of a chemical reaction. Although enzymes are grouped together on the basis of their ability to speed up chemical reactions, different types of enzymes vary greatly in their structure and function and can be formed for very specific reactions. The whole process by which an enzyme picks out its substrate or substrates works like a lock and key mechanism. An enzyme does not only select its substrate, but also ensures that the correct products are made. This ability of enzymes to channel reactions down particular pathways (*see also – Basic Strategies of Cell Metabolism*) gives a high yield of the desired product and ensures that little of the original raw material is converted into unwanted or even harmful byproducts.

The sum of all the chemical transformations that occur in cells is termed *metabolism* with the major net consequence of these transformations being the synthesis of a new cell. Whereas chemical systems, if left to themselves, always decrease in speed trying to form a neutral energy status called equilibrium whereby the slowdown becomes a random event, living organisms are highly ordered and nonrandom. In order to maintain nonrandomness, energy is required and the cells have the ability to use and transform energy which forms the central part of the life process. Cells must continuously produce and consume energy to stay alive and create their ordered structure. These two activities are generally referred to as *catabolism* and *anabolism*, respectively. Phototrophic organisms are capable to convert the sun energy into chemical energy, and chemotrophic organisms have to obtain their energy from chemical oxidations. The utilization of this chemical energy in living cells generally involves what are called *oxidation-reduction reactions*. It is these reactions which require the biocatalysts referred to earlier as enzymes. In oxidation-reduction reactions,

the electrons are moving from an electron donor to an electron acceptor during which energy is released and stored in the form of the chemical compound ATP for use in biosynthetic reactions (*see also - Cell thermodynamics and Energy Metabolism*). If the final electron acceptor is oxygen (O₂), we call these systems *aerobic*, whereas all other systems are referred to as *anaerobic*.

Although energy is required for biosynthesis, the focus here lies more on the source of carbon available. Organisms or cells which require a constant supply of organic compounds for the majority of their biosynthetic reactions are called *heterotrophs*, which include all animals, fungi, most bacteria and many algae. *Autotrophs*, on the other hand, are organisms or cells that are able to obtain all the required carbon from carbon dioxide (CO₂) and can live and grow in the absence of organic matter. One large group of autotrophs are the phototrophs, which includes plants, algae and some bacteria. An exception to this are *chemolithotrophs*. All *chemotrophs* use the energy-rich compounds synthesized by the phototrophs, representing nature's *carbon cycle*.

When looking at an overview of anabolism (biosynthesis) and catabolism, anabolism is relatively uniform in principle in all forms of life, but it is the vast diversity of catabolic or fuelling reactions which was first exploited for biotechnological purposes in form of food fermentations and industrial processes.

Strain improvement is an essential part of process development for biotechnological and pharmaceutical/medical products (*see also - Process optimization for biotechnological products*). It is a means of reducing costs by developing strains of microorganisms, traits of plant and/or animal cells with increasing productivity and yield. This may range from using cheaper raw materials for microbial processes, to pest resistance and higher yields in plants and animals. The current practice for this development has always been new strain isolation, selection and mutagenesis. Mutagenesis means the induction of inheritable changes - *mutations* - into the genetic material of any cell or organism. These mutations are caused by changes in the genotype and can be detected as a modified phenotype of the organism or mutant.

The occurrence of mutagenesis is a basic requirement of evolution and can occur in all living cells. Therefore, in microbial strain improvement and in plant cultivation, mutagenesis is used for breeding new varieties. Mutations *in vivo* may arise a number of ways: spontaneously, after induction by radiation, or use of chemical agents, resulting in various structural changes in the genome:

- a) genome mutations may cause changes in the number of chromosome sets;
- b) chromosome mutations may change the order of genes within the chromosome;
- c) gene or point mutations may result from changes in the base sequence in a gene.

The current practice of strain improvement by mutation and selection in combination with breeding by sexual and parasexual recombination plus protoplast fusion has been found to be an effective technique (*see also The Challenges of Genetic Information*).

Mutations can also be induced *in vitro* by the use of new gene technologies referred to as '*gene modification [GM]*' or '*genetic engineering*' techniques (*see also GMO-*

Technology and Malnutrition). The first experiments in which DNA fragments were joined *in vitro* and the recombinant molecules re-introduced into living cells were performed in the early 1970s. The basic information obtained in those experiments, together with numerous new discoveries in all fields of bioscience as well as in chemical, physical and computer sciences led to the development of modern genetic engineering, often referred to as the '*new biotechnology*'.

This powerful new methodology can be regarded as a set of biological, genetical, biochemical, chemical and physical procedures that greatly facilitate the localization, isolation, characterization, modification, synthesis and transfer of genetic material. The application of this new gene technology has changed nearly all areas of the biosciences and has dramatically accelerated the rate at which data can be obtained in these fields. It has also brought outstanding insights in many basic processes of living organisms and tissues.

The application of genetically modified organisms (see also *Basic Strategies of Cell Metabolism; The Importance of Microbial Culture Collections and Gene Banks in Biotechnology*) as production strains in industrial processes has revolutionised the areas of medical and industrial biotechnology. Its introduction into the plant sciences led to improved pest resistance and higher crop yields, thus revolutionising agriculture (see also *Agricultural Biotechnology*).

The introduction of gene technology into industry and agriculture and now also into animal and medical sciences is no longer a dream of the future but an integral part of present technologies.

Recombinant DNA is made by joining DNAs from different sources. The ability to do this grew out of research on the restriction enzymes, which are produced in bacteria as part of the bacterial defence against invading foreign DNAs, such as those of viruses. Restriction enzymes destroy the foreign DNA by cutting it into pieces.

They have two properties that make them extremely useful to molecular biologists. The first is their absolute specificity and the second is the ability of many to produce staggered cuts when they cleave double-stranded DNA. The specificity of restriction enzymes is already being applied as the basis of new techniques for diagnosing hereditary diseases, such as sickle cell anaemia, that are caused by structural changes in genes.

The ability to make recombinant DNA molecules opened the way to isolating and producing essentially unlimited quantities of a desired gene. Foreign DNAs could be inserted into viral DNAs that could serve as vehicles for introducing the foreign genes into bacteria or other cultural cells. The recombinant DNA will then reproduce in the cells, thus generating large quantities of the foreign genes. This reproduction of foreign genes in cells is referred to as '*gene cloning*'.

The biotechnological applications of genetic modification consists therefore offour main stages:

- 1) obtaining the gene which codes for the product to be produced,

- 2) inserting the gene into the cell,
- 3) inducing the cell to start synthesizing the foreign product and
- 4) collecting that product.

The possibility of transferring genes from one organisms to another or from one cell to another is an alluring prospect since genetic engineering or gene modification could reduce the cost and increase the supply of an enormous range of materials now used in medicine, agriculture and industry.

3. Metabolism

Metabolic processes are concerned with all those biological or chemical reactions which can be carried out by the cell. It is essential for the biotechnologist to fully understand these basic metabolic processes, as every present and future biotechnological industry can be economically feasible only if full advantage is taken of the cell's capacity to convert substrate into the desired product.

Metabolism is the intricate interplay between anabolism and catabolism via the regulatory mechanisms to observe the thermodynamic laws of nature.

The interconnections between plant cells, animal cells, and microbial cells can be visualized best in the geochemical cycles of matter in nature, the carbon, nitrogen, phosphate etc cycles (*see also - Environmental Biotechnology*).

Without a thorough knowledge of cell metabolism, little improvement in the field of biotechnology is possible. It is essential to fully exploit the genotype , which is only possible through a good understanding of cellular metabolism.

3.1 Thermodynamics

The most fundamental property of living cell systems is their ability to utilise and transform energy involving thousands of individual and enzyme-catalysed chemical reactions (*see also – Cell Thermodynamics and Energy Metabolism*).

Since every chemical reaction involves a loss or gain of electrons, the amount of energy released or used depends on the oxidation-reduction potential difference or distance between the electron donor [oxidised compound] and electron acceptor [reduced compound].

In order to maintain its integrity, gain and loss of energy must be balanced via a controlled flow [electron transport] and energy transformation [ADPATP], which follows the laws of thermodynamics.

On the basis of electron donor and electron acceptor availability , four modes of energy production are recognized:

- 1) photosynthesis,
- 2) aerobic respiration,
- 3) anaerobic respiration and
- 4) fermentation.

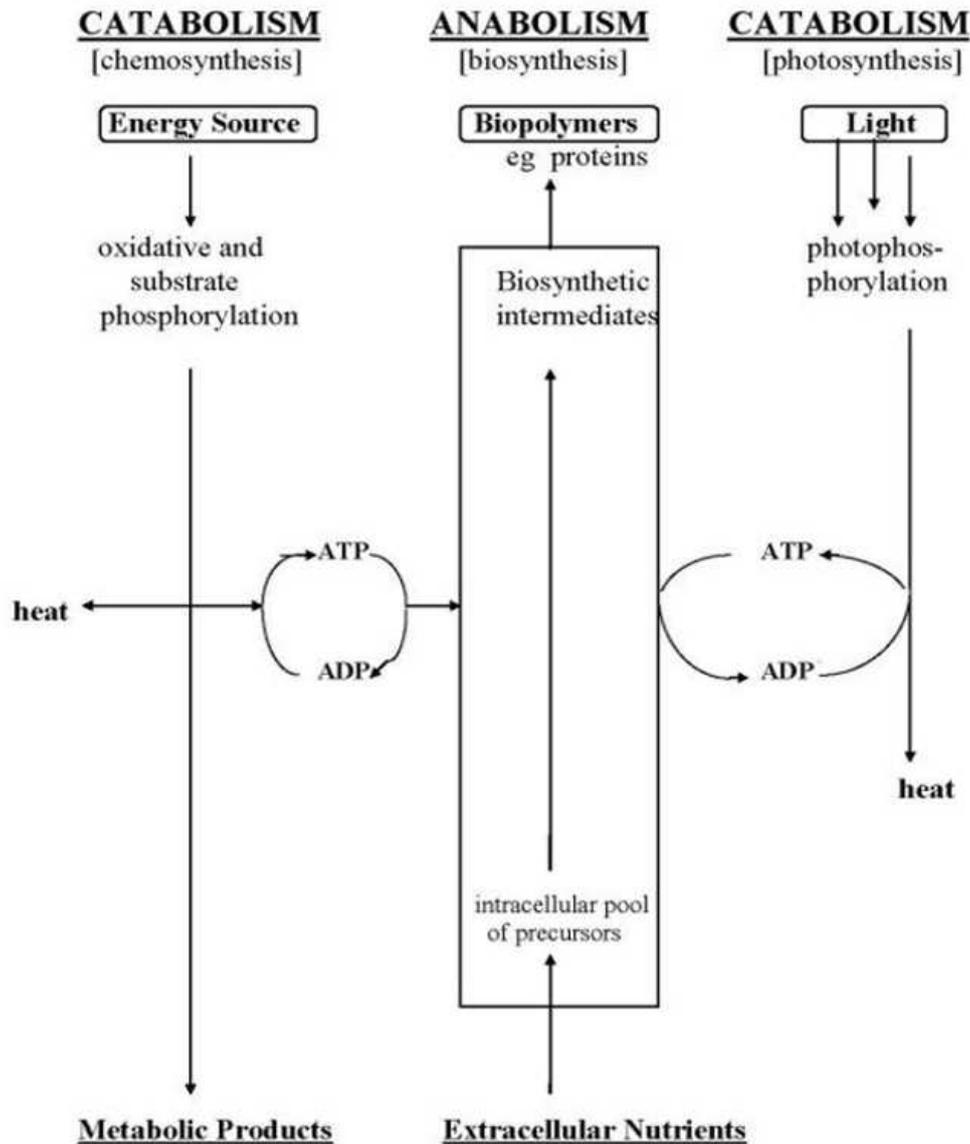


Figure 1: Generalised scheme for metabolic energy formation and usage [adapted from Doelle 1994b]

The energy transformations are also vital for the transport of solutes along pH and electrical gradients across the otherwise impermeable cellular membrane.

Cell metabolism consists of thousands of individual chemical and enzyme-catalysed chemical reactions. These chemical reactions in living organisms occur in characteristically organised sequences, called *metabolic pathways* (see also - *Basic Strategies of Cell metabolism*) There are two main types of metabolic pathways :

- pathways which lead from large [low oxidative state] to smaller molecules [high oxidative state], which are called catabolic pathways or catabolism
- pathways which lead from small [high oxidative state] to large molecules [low oxidative state] essential for the formation of cellular material, which are referred to as anabolic or biosynthetic pathways or anabolism.

The main concept of catabolism is therefore to provide the cell with small molecules or precursors suitable for biosynthesis of all major chemical constituents of the living cell and with reductant and energy as well to carry out these *endergonic* and reducing reactions leading towards compounds of low oxidative state (Figure 1). Whereas all catabolic pathways are oxidative and thus energy-producing, the biosynthetic pathways are reductive and energy-consuming. Metabolism consists therefore entirely of energy transformation and transfer mechanisms, which are based on thermodynamics.

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Biographical Sketches

Horst W.Doelle, born in 1932, studied biology at the University of Jena [1950-1954]. He studied for his doctorate at University of Goettingen [1955-1957] on antibiotic production. After receiving his doctorate, he worked in the Wine and brewing industry in Germany before taking up an appointment with CSIRO in Australia in 1960. After 4 years wine research, he took up the challenge to build up microbial physiology

and fermentation technology at the Department of Microbiology at the University of Queensland in Brisbane. He received his Doctor of Science in 1976 and his Doctor of Science honoris causa in 1998. He participated and conducted numerous training courses in developing countries. After 29 years teaching he retired in 1992. His research area was regulation of anaerobic/aerobic metabolism, microbial technology [*Zymomonas ethanol* technology] and socio-economic biotechnology using microorganisms for waste management.

Monica Wilkinson nee Doelle was born in 1963 in Griffith, NSW. She attended St Peters Lutheran College in Brisbane and studied microbiology at the University of Queensland. She obtained her Bachelor of Science in 1985, majoring in Microbiology. In 1988 she obtained her MSc Qualifying degree and in 1991 her PhD in Microbiology. She received in 1996 her Diploma of Education. For fourteen years, Monica worked within the microbiology/biotechnology industry and published many papers in the area of her work. She has also been involved in teaching at many different levels, from High School to University level, as well as work placed training programmes. She always worked with the aim to equip others to meet challenges, encourage enthusiasm in an ever-changing industry and society.