

BASIC STRATEGIES OF CELL METABOLISM

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Keywords: metabolism, catabolism, anabolism, biosynthesis, regulation, carbohydrates, proteins, fats, single carbon compounds, starch, cellulose, amino acids, fatty acids, allostery, RNA, DNA, autotrophy, heterotrophy, TCA cycle, ethanol, methane, carbon dioxide fixation

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Summary

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.

For the biotechnological evaluation of the usefulness of a microbial process, for example, the stoichiometry of microbial metabolism together with its regulatory mechanisms can be a very useful tool, since stoichiometric equations describing the consumption of a substrate and the formation of biomass and products are always required. With a knowledge of the basic concept of catabolism, it is possible to explore the enormous versatility of the microorganism, plant, algae, mammalian in sustaining and maintaining life under unfavourable conditions. It should always be remembered that organic endproduct formation is a result of reduced energy and biomass formation. This exchange of energy and biomass for endproducts leads to major [eg primary] or minor [secondary] products.

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, no 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.

1. Introduction

In order to grow, cells have to build up a vast array of chemical substances of which they are composed of. These substances, often quite complex, are synthesized from simpler molecules by processes called *anabolism*. If the simple molecule is CO₂, one refers to *carbon assimilation* and the cell is said to be *autotrophic* [eg plants, algae, bacteria]. The majority of microbial cells in nature uses simple molecules from the degradation of organic molecules and thus are referred to as *heterotrophic*.

The biosynthetic (anabolic) reactions involved in cellular growth are often energy-requiring and ATP formed during photosynthesis or chemosynthesis is used up during

these biosynthetic reactions. **Chemoheterotrophic** cells generate therefore the required energy during the oxidation/utilization of organic compounds. These reactions involve oxidation-reduction processes accompanied by the release of energy, some of which is conserved in ATP [see also - *Cell Thermodynamics and Energy Metabolism*]. The particular compound may be oxidised either completely to CO₂ in the case of **aerobic respiration** or only partially in the case of **anaerobic respiration and fermentation** to lower molecular carbon compounds. The processes involved in the oxidation of compounds are collectively called **catabolism**. Catabolic and anabolic (biosynthetic) reactions are referred to as **metabolic reactions**. The term **metabolism** therefore refers to the whole array of oxidative (degradation) and reductive (biosynthetic) reactions taking place within the cell.

Although energy is required in certain key biosynthetic reactions, the focus of biosynthesis is not on energy, but rather on carbon and on the intermediates occurring in the build-up of cell constituents from simple starting materials. These intermediates are often formed as a result of catabolism, but also serve as starting materials in biosynthetic reactions. Some of the starting compounds for biosynthesis are formed by simple depolymerisation of large molecules. For example, protein may be hydrolysed to amino acids, which can be directly used in new protein biosynthesis provided the cell possesses the particular transport systems for these amino acids.

The relationships between catabolism and anabolism occur not only at the common intermediates. Certain pathways play dual roles, as they function in both anabolism and catabolism. For instance, the tricarboxylic acid cycle, as will be demonstrated later, is involved not only in the oxidation of pyruvate and acetyl-CoA to carbon dioxide for energy production, but also in the generation of a number of intermediates such as succinyl-CoA, oxalacetate and ketoglutarate, which serve as starting points for the synthesis of amino acids, porphyrins and other compounds necessary for growth. A pathway that serves the dual function of catabolism and anabolism is called an **amphibolic** pathway. Many pathways are amphibolic. Thus ketoglutarate may be oxidised to succinate, leading to energy generation, or it may be converted to the amino acid glutamate for use in protein biosynthesis. Obviously the cell has to make a choice. A given molecule at a given moment may go to catabolic or anabolic sequences; it cannot do both simultaneously. However, if one has a cyclic mechanism like the TCA cycle, any compound taken up for biosynthetic reactions would endanger further functioning of the cycle as links in the cycle would be deleted. This deficiency is overcome by replacement of oxalacetate by auxiliary synthetic reactions that come out of the main cycle. Reactions of this type have been grouped under the term **anaplerotic**, meaning 'replenishing' reactions.

One key regarding the contrasts between anabolic and catabolic reactions is that even where the same reactants are involved, the enzymes acting in anabolic reactions are often different from those involved in catabolic reactions.

In **eukaryotes**, a further difference between anabolism and catabolism is in the cellular localisation of the processes. Many catabolic reactions are localised in the mitochondria and microbodies, whereas anabolic reactions are primarily cytoplasmic. At least one advantage of having separate sites for catabolic and anabolic reactions is that both can

occur at the same time without confusion.

In *prokaryotes*, where compartmentation is less structured, control of reactions occurs mainly at the level of single enzymes.

2. Polymer hydrolysis

Organic substances in nature are in abundance in the form of polymers. Under this term we understand molecules consisting of basic monomeric units linked together. For example, if the monomeric structure consists of carbohydrates, one uses the term *polysaccharides*. These polymers cannot penetrate the cell membrane and must be broken down first into small transportable molecules, which in many cases are the monomers. This enzymatic process of cutting the chains into their basic components is generally referred to as *hydrolysis* and the corresponding enzymes are therefore called *hydrolytic enzymes* or *hydrolases*. The action of these hydrolytic enzymes is of extreme importance and often does not underlie specific control systems, but exhibit reaction rates completely independent from the monomeric cell uptake or transport rates. They play a significant role in the macroscopic degradations such as food spoilage and waste treatment apart from the desirable ageing of meat, curing cheese, preventing beer haze and many other processes. The isolation and purification of these enzymes is a large commercial enterprise.

There are two polymers which become of increasing interest because of their abundant availability in nature: **starch** and **cellulose**. Both polymers are *polysaccharides*, which means their monomers are sugars, or in this specific case, *glucose*. It is of interest to realise that although these two polymers are formed from the same monomer, they possess vastly different properties. Starch is readily digestible by many microorganisms and is a prime energy source, whereas cellulose, the key constituent of plant cell walls, is more difficult to digest whether in its pure form or linked with the heteropolymer lignin.

2.1. Starch hydrolysis to glucose

The chemical structure of starch is essentially the same throughout nature. The naturally occurring starch is a mixture of two polysaccharides, both of which are polymers of glucose. The major component is **amylopectin** with a branched structure and the minor component is **amylose**, which is a linear macromolecule. **Amylose** is a flexible, linear chain molecule of 500 or more glucose units and gives a blue colour with iodine. The glucose units are joined by *alpha-1,4-glycosidic* linkages. The linear portion of amylopectin is identical to amylose, but every 20-30 glucose units a branching occurs by an *alpha-1,6-glycosidic* linkage [see also - *Biotechnology of Archae*]. Amylopectin gives a red to purple colour with iodine.

Starch hydrolases degrade the polysaccharide to soluble, low molecular weight products such as glucose. These enzymes were originally called *diastases*, but now they became **amylases**:

amylopectin and amylose completely to glucose and is capable to hydrolyse the α -D-(1 \rightarrow 4) as well as the α -D-(1 \rightarrow 6) glycosidic bond.

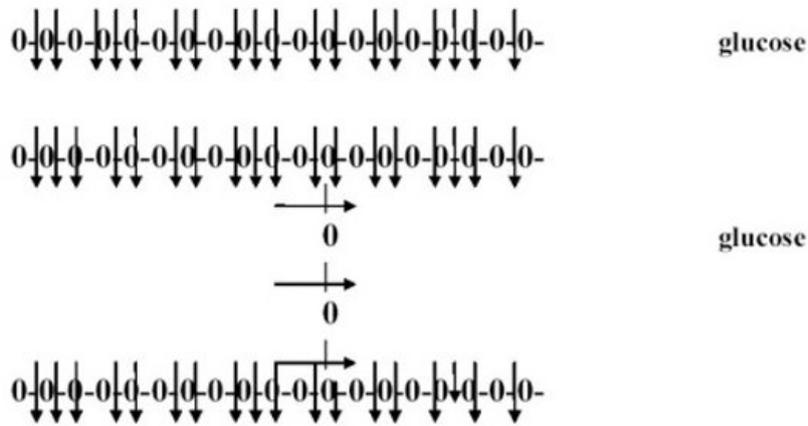


Figure 3: Starch hydrolysis using the enzyme amyloglucosidase

Amongst the three different amylases known, only one is capable to break both types of glycosidic linkages and this is glucoamylase [EC 3.2.1.3]. Only this particular enzyme is able to convert all the starch into glucose without any other product being formed.

2.2. Cellulose

Cellulose is the largest biological component of the renewable resources available on earth. However, most of the cellulose occurs in a lignocellulose complex containing large fractions of crystalline cellulose. It is the enzyme **ligninase**, which separates the lignin component from the cellulose. This enzyme occurs only in mushrooms. The resistance of these natural forms of cellulose to chemical and microbial attack has so far limited its use. The hydrolysis of cellulose by cellulolytic enzymes is therefore under intensive studies in order to develop a process for the production of sugars. Although cellulose consists of glucose units as does starch, the bonding arrangement of these glucose molecules is quite different. In cellulose, the glucose units are linked by β -(1 \rightarrow 4) linkages. The hydrolysis products are therefore glucose and cellobiose. At least four enzymes are known to be involved in the cellulose to glucose conversion:

1. **cellobiohydrolase**, which splits cellobiose from the non-reducing end of the cellulose chain;
2. **exoglucanase**, which splits glucose from the non-reducing end of the cellulose chain;
3. **cellobiase**, which hydrolyses cellobiose to glucose;
4. **endoglucanase**, which hydrolyses long polymers into oligosaccharides.

Our present knowledge on cellulose degradation is still relatively scanty, but it is known that cellobiose is the first product of cellulose hydrolysis and is a potent inhibitor of cellulose hydrolysis (Figure 4). It is therefore important that the enzyme cellobiase is present to remove cellobiose from the medium. The complexity of the system certainly

awaits further intensive research, in particular the removal of the heteropolymer lignin from the lignocellulose, as cellulose in nature is surrounded by a lignocellulosic ring enclosure. So far, only certain fungi species [eg. mushrooms] are known to be able to split this ring .

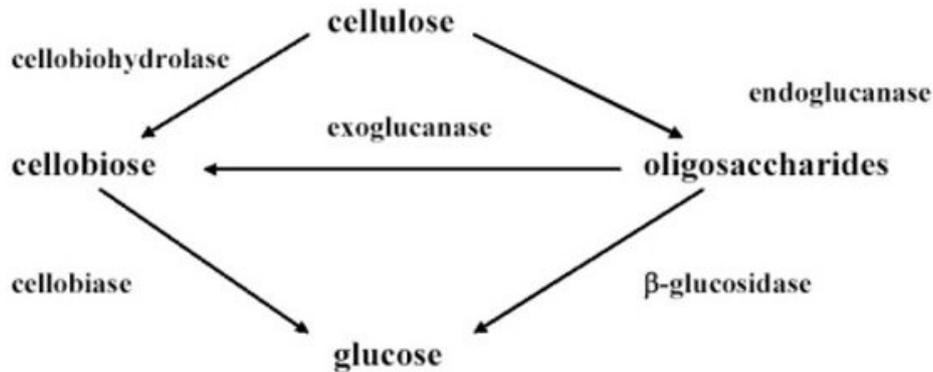


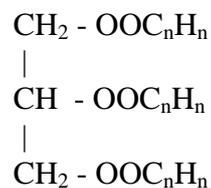
Figure 4: Cellulose hydrolysis to glucose

2.3. Proteins

The ability to break down proteins is very similar to the above described polymer degradation. In the case of proteins, the enzymes involved are *proteolytic enzymes*, **proteases and proteinases**, which are classified according to their attacks on the individual proteins and polypeptide chains. They hydrolyse peptides and are able to break down the chain, liberating di- or tripeptides, which the di- and tripeptidases utilise further forming free amino acids. These monomers are now able to diffuse or be transported into the cell.

2.5. Fats

Fats are another major carbon source for microorganisms. The majority of fats are simply *triglycerides*, that is glycerol substituted fatty acids:



Similar to the cellulose, starch or protein polymers, fats have to be converted into monomeric substrates, which are fatty acids and glycerol. This hydrolysis is carried out by **lipases** outside the cell. These lipases separate the fatty acids from the glycerol moiety resulting in glycerol plus 3 fatty acids. Under the term lipase one understands a conglomerate of different hydrolytic enzymes or *esterases*, which specifically act upon the different glycerol-substituted fatty acids.

The lipase action can occur with a simultaneous fatty acid activation to its CoA-derivative, as it occurs in the prokaryotes or can simply lead to a fatty acid followed by

an acylation involving **carnitine**, which serves as a carrier of acyl-groups into and out of mitochondria, as it occurs in the eukaryotes including mammalian cells.

In bacteria, the first attack on the triglyceride is catalysed by a *diglyceride acyltransferase*, whereby the fatty acid in the 3-position of the glycerol is released and immediately converted into its CoA derivative. The so formed alcohol group is then phosphorylated before the other two fatty acids are released. This phosphorylation step is catalysed by the *phosphatidate phosphorylase* (EC 3.1.3.4), which incorporates 1 molecule inorganic phosphate into the diglyceride, thus forming phosphatidic acid. (Figure 5).

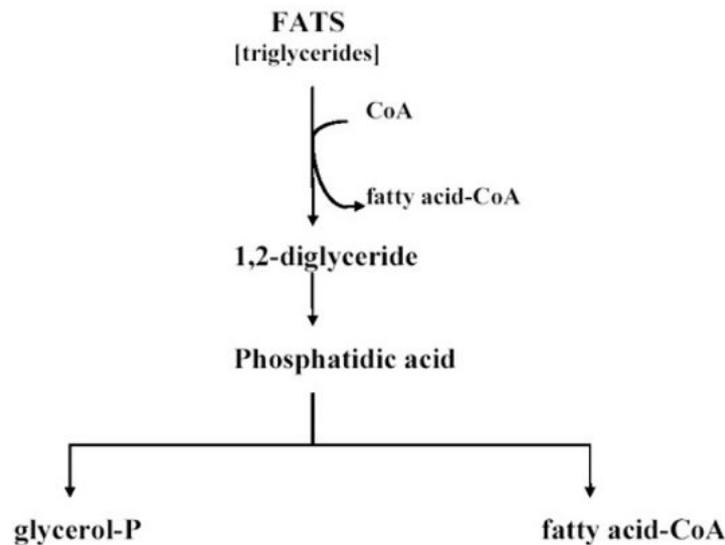


Figure 5: Fat hydrolysis

The two residual fatty acids on the phosphatidic acid molecule are now removed and transformed to their respective CoA-derivatives by *glycerophosphate acyltransferase* (EC 2.3.1.15). The endproducts of these reactions are therefore the monomers glycerophosphate plus 3 fatty acid CoA esters. These compounds can now be transported into the cell.

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Biographical Sketch

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.