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Springer-Verlag New York Heidelberg Berlin

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With 161 figures.

Library of Congress Cataloging in Publication Data

Gottschalk, Gerhard.
Bacterial metabolism.
(Springer series in microbiology)
Includes bibliographical references and index.
1. Microbial metabolism. 2. Bacteria—Physiology.
I. Title. DNLM: 1. Bacteria—Metabolism.
QW52.3 G687b
QR88.G67 589.9'01'33 78-7880

Printed in the United States of America.
9 8 7 6 5 4 3 2 1

ISBN 0-387-90308-9 Springer-Verlag New York
ISBN 3-540-90308-9 Springer-Verlag Berlin Heidelberg

Preface

This book has been written for students who are taking a course in bacterial metabolism. I hope, however, that scholars will also find it useful either as a help in teaching bacterial metabolism or as a review on the special aspects of metabolism in bacteria.

The concept of this book results from my experience in teaching bacterial metabolism. In the first chapters the principal reactions of the energy and biosynthetic metabolism have been discussed using *Escherichia coli* as a model organism. Then the diversity of aerobic metabolism has been outlined. Following a brief description of the regulation of the level and the activity of enzymes in bacteria the characteristic features of fermentative, chemolithotrophic and phototrophic metabolism have been discussed. Finally, the last chapter has been devoted to nitrogen fixation. Throughout the text I have tried not only to describe metabolic pathways and enzyme reactions but also to elucidate the physiology of the microorganisms which carry out all these metabolic reactions.

Two comments regarding the formulas used in this book are necessary. Organic acids are usually called after the names of their salts which are shorter (formate for formic acid, pyruvate for pyruvic acid). However, in schemes and figures the formulas of the free acids are given. Furthermore, it should be pointed out that $NADH_2$ and $NADPH_2$ and not NADH and NADPH are used as abbreviations for reduced nicotinamide-adenine dinucleotide and reduced nicotinamide-adenine dinucleotide phosphate, respectively. This has been done as these compounds are two electron carriers and redox reactions involving these carriers are thus easier to formulate.

I am particularly indebted to Joan Macy, Lynne Quandt, Jan Andreesen and Peter Hillmer for reading the manuscript, for their criticisms and their suggestions, and I thank Ute Gnass for typing the manuscript and for her

invaluable help with the indexing and with the preparation of the figures. Finally, I am grateful to the publishers for their patience, willing help, and cooperation.

Göttingen, 1978 GERHARD GOTTSCHALK

Contents

Chapter 1

Nutrition of Bacteria

Bacteria, like all other living organisms, require certain nutrients for growth. These nutrients must contain those chemical elements that are constituents of the cellular materials and that are necessary for the activity of enzyme and transport systems. In addition, the nutrients must provide the organisms with materials for the production of biologically utilizable energy.

1. Major and Minor Bio-Elements

Only a small number of the elements of the periodic system are required by organisms in relatively high concentrations ($> 10^{-4}$ M). These ten major bio-elements and some of their functions are presented in Table 1.1. Carbon, oxygen, hydrogen, and nitrogen are the main constituents of the organic compounds occurring in organisms. Sulfur is required for the synthesis of the amino acids cysteine and methionine and of a number of coenzymes. Phosphorus is present in nucleic acids, phospholipids, teichoic acids, and in nucleotides such as ATP, GTP, NAD, and FAD. The remaining four major bio-elements are metal ions, which are required as cofactors for enzyme activity and as components of metal complexes. Most of the biologically active phosphate esters are, for instance, present in the cell as magnesium complexes. The phospholipoproteins of bacterial cell walls are also chelated with magnesium ions.

Exoenzymes such as amylases and proteases are calcium proteins, and calcium dipicolinate is an important component of endospores. Ferrous and ferric ions are present in redox carriers such as cytochromes and iron-sulfur proteins.

Table 1.1. The ten major bio-elements, their sources, and some of their functions in microorganisms

element	source	function in metabolism
C	organic compounds, CO_2	main constituents of cellular material
O	O_2, H_2O, organic compounds, CO_2	
H	H_2, H_2O, organic compounds	
N	NH_4^+, NO_3^-, N_2, organic compounds	
S	SO_4^{2-}, HS^-, S^0, $S_2O_3^{2-}$, organic sulfur compounds	constituent of cysteine, methionine, thiamin pyrophosphate, coenzyme A, biotin, and α-lipoic acid
P	HPO_4^{2-}	constituent of nucleic acids, phospholipids, and nucleotides
K	K^+	principal inorganic cation in the cell, cofactor of some enzymes
Mg	Mg^{2+}	cofactor of many enzymes (e.g., kinases); present in cell walls, membranes, and phosphate esters
Ca	Ca^{2+}	cofactor of enzymes; present in exoenzymes (amylases, proteases); Ca-dipicolinate is an important component of endospores
Fe	Fe^{2+}, Fe^{3+}	present in cytochromes, ferredoxins, and other iron-sulfur proteins; cofactor of enzymes (some dehydratases)

Besides these ten major bio-elements, organisms require a number of others in small amounts (Table 1.2). Zinc and manganese ions are essential for all microorganisms. Zinc is especially important because RNA and DNA polymerase are zinc-metalloproteins. Sodium chloride is required by halophilic microorganisms in high concentration. This, however, is the exception. Most microorganisms have little use for sodium and chloride ions. Specific functions can be assigned to the other metals listed in Table 1.2. Molybdoproteins play an important role in nitrogen metabolism and in formate oxidation. Xanthine dehydrogenase also contains molybdenum. Of the selenoproteins listed in Table 1.2, the glycine reductase contains selenium in the form of selenocysteine. Cobalt is required by all organisms that perform B_{12}-dependent reactions. Copper is present in a number of

Table 1.2. Minor bio-elements, their sources, and some of their functions in microorganisms

element	source	function in metabolism
Zn	Zn^{2+}	present in alcohol dehydrogenase, alkaline phosphatase, aldolase, RNA and DNA polymerase
Mn	Mn^{2+}	present in bacterial superoxide dismutase; cofactor of some enzymes (PEP carboxykinase, re-citrate synthase)
Na Cl	Na^{+} Cl^{-}	required by halophilic bacteria
Mo	MoO_4^{2-}	present in nitrate reductase, nitrogenase, and formate dehydrogenase
Se	SeO_3^{2-}	present in glycine reductase and formate dehydrogenase
Co	Co^{2+}	present in coenzyme B_{12}-containing enzymes (glutamate mutase, methylmalonyl-CoA mutase)
Cu	Cu^{2+}	present in cytochrome oxidase and oxygenases
W	WO_4^{2-}	present in some formate dehydrogenases
Ni	Ni^{2+}	present in urease; required for autotrophic growth of hydrogen-oxidizing bacteria

enzymes transferring electrons from substrates to oxygen. Finally, tungsten and nickel are needed by microorganisms in some rare cases.

In nature, most of the bio-elements occur as salts, and they are taken up by the organisms as cations and anions, respectively. A greater diversity of compounds utilized by microorganisms is only observed with respect to the first five elements of Table 1.1: sulfur, nitrogen, oxygen, hydrogen, and carbon.

Sulfur is normally taken up as sulfate, reduced to the level of sulfide, and then used for biosynthetic purposes. Certain groups of bacteria, however, depend on the availability of reduced sulfur compounds. Some methane bacteria grow only in the presence of hydrogen sulfide as sulfur source. Thiobacilli and a number of phototrophic bacteria require sulfide, elemental sulfur, or thiosulfate as electron donor.

Nitrogen is required in large quantities because it amounts to approximately 10% of the dry weight of bacteria. It occurs naturally in the form of ammonia, nitrate, nitrite, nitrogen-containing organic compounds, and molecular nitrogen. The preferred source of nitrogen is ammonia, which can be utilized by practically all microorganisms. Nitrate is also taken up and used by many microorganisms but not by all. Before it can be incorporated into organic substances it has to be reduced to ammonia. Nitrite is the product of the nitrate–nitrite respiration and of the metabolic activities

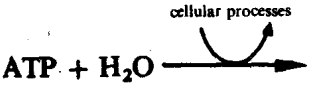
of *Nitrosomonas* and related species. A number of organisms reduce it to ammonia or N_2. Alternatively, nitrite can be oxidized to nitrate by *Nitrobacter* species. Several bacteria are able to fix molecular nitrogen and to reduce it to ammonia. This capacity is found only in certain prokaryotes but not in eukaryotes. Finally, organic compounds serve as nitrogen sources for many microorganisms. Usually these compounds are degraded such that ammonia becomes available for biosyntheses.

Carbon, hydrogen, and oxygen can be utilized by bacteria in the form of organic and inorganic compounds. Among the inorganic compounds used are CO_2, H_2, H_2S, NH_3, H_2O, O_2, NO_3^-, and SO_4^{2-}. On earth, not a single organic compound formed by organisms is accumulated. This implies that all of them are degradable. Microorganisms play an important role in this degradation. Their versatility has led to the formulation of the "doctrine of microbial catabolic infallibility," meaning that every naturally occurring carbon compound is used by some microbe.

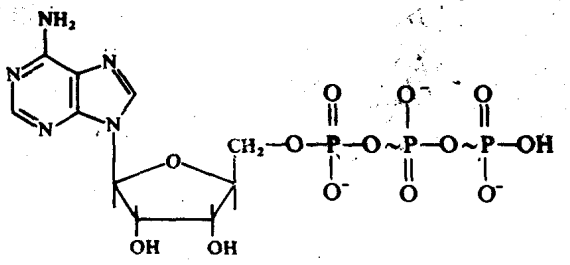
The metabolism of carbon-, hydrogen-, and oxygen-containing compounds is not only important because these elements are the main constituents of the cell. These compounds are important substrates for the energy production in microorganisms.

II. The Two Basic Mechanisms of ATP Synthesis

The principal carrier of biologically utilizable energy is adenosine-5'-triphosphate (ATP), and all energy-requiring processes in living cells are directly or indirectly coupled to the conversion of ATP to adenosine-5'-diphosphate (ADP) and inorganic phosphate (P_i):



ATP contains two phosphate bonds with a high free energy of hydrolysis. The bonds are often symbolized by the squiggle "~":



Because of the high-energy phosphoryl bonds, ATP is an excellent phosphorylating agent, and it is used as such in a large number of reactions by all organisms. At the expense of ATP, intermediates of cell metabolism are activated for further reactions, such as condensations, reductions, and cleavages. Glutamine, for instance, can be synthesized from glutamate and ammonia only if a phosphorylated intermediate is formed. The reaction is, therefore, connected with the formation of ADP and P_i from ATP:

$$\text{glutamate} + NH_3 + \text{ATP} \longrightarrow \text{glutamine} + \text{ADP} + P_i$$

The high potential of group transfer of the AMP and the ADP group is also taken advantage of in a number of reactions; amino acids are activated by their conversion into the corresponding AMP derivatives with ATP, and AMP is released in the formation of aminoacyl-transfer-RNA:

$$\text{amino acid} + \text{ATP} \longrightarrow \text{aminoacyl-AMP} + \text{pyrophosphate}$$
$$\text{aminoacyl-AMP} + \text{transfer-RNA} \longrightarrow \text{aminoacyl-transfer-RNA} + \text{AMP}$$

The enzyme adenylate kinase catalyzes the phosphorylation of AMP to ADP with ATP; pyrophosphate (PP_i) is hydrolyzed to inorganic phosphate by pyrophosphatase so that the end products of this reaction series are also ADP and P_i:

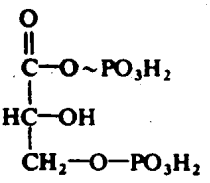
$$\text{AMP} + \text{ATP} \xrightarrow{\text{adenylate kinase}} 2\,\text{ADP}$$

$$PP_i + H_2O \xrightarrow{\text{pyrophosphatase}} 2\,P_i$$

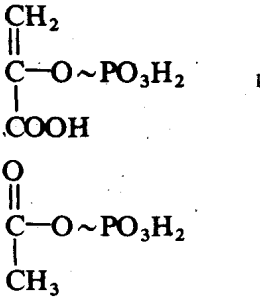
ADP and P_i are thus the principal products of the energy expenditure in metabolism, and the generation of ATP from ADP and P_i is a vital process of all living organisms. There are two basic mechanisms of ATP generation: electron transport phosphorylation and substrate-level phosphorylation.

Electron transport phosphorylation refers to a mechanism in which the flow of electrons from donors with a negative redox potential to acceptors with a more positive redox potential is coupled to the synthesis of ATP from ADP and P_i. Systems in which electron transport phosphorylation occurs are the respiratory chains and the photosynthetic apparatus.

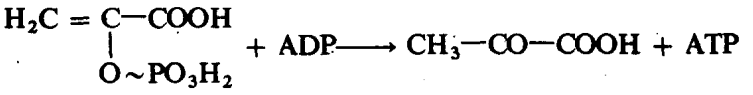
Substrate-level phosphorylation is the second mechanism of ATP generation. During the degradation of organic substrates a small number of intermediates is formed containing high-energy phosphoryl bonds. Intermediates of this kind are:



1,3-bisphosphoglycerate



The further metabolism of such organic ~P compounds is coupled to the transfer of the phosphate group to ADP, and this kind of ATP synthesis is called substrate-level phosphorylation:

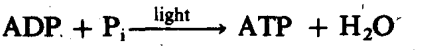


III. Nutrients as Energy Sources

It has already been mentioned that the function of the nutrients is not only to provide the organisms with the bio-elements. Nutrients are also required as energy sources—as fuel for the production of ATP. Various energy sources are available in nature and are taken advantage of by microorganisms, but they cannot be used by every bacterium, and it has become useful to group bacteria on the basis of their characteristic energy source. Organisms using light as energy source are called **phototrophs** (Greek phos = light, trophe = nutrition). If ATP comes from chemical reactions, the organisms which carry out this type of energy metabolism are called **chemotrophs.**

A. Phototrophy

Phototrophs contain a photosynthetic apparatus that enables them to convert light energy into the high-energy phosphate bonds of ATP:



The carbon source commonly used by phototrophs is CO_2, and organisms that derive most of their cell carbon from CO_2 are called **C-autotrophs** (Greek autos = self; autotroph = self-feeding). Thus, phototrophic bacteria are C-autotrophic organisms, and when growing with CO_2, they require an electron donor for the reduction of CO_2 to the level of cell material. It is apparent from Table 1.3 that, as in plants and in blue-green bacteria, the electron donors used are frequently inorganic compounds. Phototrophic bacteria employ molecular hydrogen or reduced sulfur compounds, and

Table 1.3. The two types of phototrophic metabolism

type	electron donor	carbon source	examples
photolithotrophy	H_2O	CO_2	plants, blue-green bacteria
	H_2S, S^0, H_2	CO_2	*Chromatiaceae*, *Chlorobiaceae*
photoorganotrophy	organic substrates	organic substrates	*Rhodospirillaceae*

when doing so they are called **photolithotrophs** (Greek lithos = stone). A number of phototrophic bacteria can also grow in the light with organic substrates such as succinate or acetate. Under these conditions the source of any electrons used in reduction reactions is an organic substrate, and the bacteria grow then as **photoorganotrophs**. Clearly, the main source of cell carbon is then the organic substrate and not CO_2, and the organisms grow as **C-heterotrophs** (Greek heteros = the other; heterotroph = feeding on others). It follows that the terms C-autotroph and C-heterotroph reflect the nature of the carbon source, whereas the terms lithotroph and organotroph describe the nature of the electron donor used.

B. Chemotrophy

Most bacteria gain ATP by chemical reactions. These are commonly oxidation-reduction reactions, which means that one substrate is reduced at the expense of a second one:

$$X_{red} + A_{ox} \longrightarrow X_{ox} + A_{red}$$
$$ADP + P_i \quad ATP + H_2O$$

Higher organisms can only use organic substrates as electron donors (X_{red}) and oxygen as electron acceptor (A_{ox}) and it is a specialty of the bacterial energy metabolism that, alternatively, other donors and acceptors can be employed. Here, A_{ox} may stand for oxygen, nitrate, sulfate, CO_2, or an organic compound, and X_{red} for an inorganic or an organic compound. By analogy to the nutritional classification of the phototrophs, bacteria which employ an organic compound as electron donor are called **chemoorganotrophs**. This group includes aerobes and anaerobes. The anaerobes, as is apparent from Table 1.4, use either nitrate, sulfate, or organic substrates as electron acceptors. Thus, organisms carrying out fermentations—such as the clostridia and lactic acid bacteria—belong to this group.

Chemolithotrophs use inorganic electron donors such as hydrogen,

Table 1.4. The two types of chemotrophic metabolism

type	electron donor	electron acceptor	carbon source	examples
chemoorgano-trophy	organic substrate	O_2	organic substrate	pseudomonads, bacilli
	organic substrate	NO_3^-	organic substrate	*Bacillus licheni-formis*
	organic substrate	SO_4^{2-}	organic substrate	sulfate reducers
	organic substrate	organic substrate	organic substrate	clostridia, lactic acid bacteria
chemolitho-trophy	H_2	O_2	CO_2	hydrogen-oxidizing bacteria
	H_2S	O_2	CO_2	thiobacilli
	H_2S	NO_3^-	CO_2	*Th. denitrificans*
	Fe^{2+}	O_2	CO_2	*Th. ferrooxidans*
	NH_3	O_2	CO_2	*Nitrosomonas*
	NO_2^-	O_2	CO_2	*Nitrobacter*
	H_2	CO_2	CO_2	methanogenic bacteria
	H_2	CO_2	CO_2	*Acetobacterium*

hydrogen sulfide, ferrous ions, nitrite, or ammonia. These compounds are oxidized with oxygen to water, sulfate, ferric ions, and nitrate, respectively, and these exergonic reactions are coupled to the formation of ATP from ADP and P_i:

$$H_2 + \tfrac{1}{2}O_2 \longrightarrow H_2O$$
$$ADP + P_i \quad ATP + H_2O$$

Some organisms, e.g., *Thiobacillus denitrificans*, can replace oxygen by nitrate.

Although the methanogenic and acetogenic bacteria are quite different from all other chemolithotrophs, because they are strictly anaerobic organisms, they belong to this nutritional group. They gain ATP by reduction of CO_2 to methane or acetate with molecular hydrogen. Thus, only inorganic substrates are used for energy production.

Clearly, chemolithotrophs gain ATP without metabolizing an organic compound. Their carbon source is usually CO_2, and they are, therefore, C-autotrophs. However, many chemolithotrophs are not restricted to this kind of energy metabolism. Hydrogen-oxidizing bacteria, for instance, can grow as chemoorganotrophs (aerobically with carbohydrates or organic acids) under appropriate conditions. They are, therefore, called facultative

chemolithotrophs (all hydrogen-oxidizing bacteria, some thiobacilli). Species (*Nitrosomonas*, *Thiobacillus thiooxidans*), which are unable to grow in the absence of their inorganic electron donors, are called obligate chemolithotrophs.

IV. Growth Factor Requirements of Bacteria

So far it has been presumed that microorganisms themselves are able to synthesize all organic compounds required for growth. In fact, there are C-autotrophic bacteria that derive their cell carbon from CO_2 exclusively (e.g., *Alcaligenes eutrophus* and *Nitrobacter winogradskyi*) and there are also C-heterotrophs that grow on simple carbon sources such as glucose (e.g., *Escherichia coli*, *Bacillus megaterium*, and *Clostriaium pasteurianum*). However, a number of bacteria lack the ability to synthesize all the organic compounds needed for growth and depend on certain growth factors. These factors can be combined to form three groups:

1. vitamins and related compounds, required in small amounts
2. amino acids
3. purines and pyrimidines

Number and kind of growth factors, which must be present in the growth medium, differ among bacteria. Lactic acid bacteria require practically all amino acids, purines, pyrimidines, and vitamins for growth. Their biosynthetic capacity is rather limited. Common among microorganisms are requirements for vitamins and related compounds. Some of them and their functions in metabolism are summarized in Table 1.5.

The exact growth factor requirements are not known for all microorganisms, and microbiologists add yeast extract and peptone to the growth media as complex and cheap sources of these factors. Synthetic media—media of known composition—are used for special purposes provided the requirements of a particular organism are known. *Clostridium kluyveri* grows in a medium supplemented with biotin and *p*-aminobenzoic acid. To the media for phototrophic bacteria, a vitamin solution is added containing nicotinic acid, thiamin, *p*-aminobenzoic acid, biotin, and vitamin B_{12}. Some organisms exhibit special requirements. A medium for *Haemophilus* species must contain hemin for cytochrome biosynthesis and also NAD. *Bacteroides* species require hemin. *Methanobacterium ruminantium* grows only if coenzyme M (2-mercaptoethanesulfonic acid) and 2-methyl-*n*-butyric acid are present. These few examples document that microoganisms may exhibit various defects in their biosynthetic machinery, and that growth factors are important for many of them.