METABOLIC DIVERSITY IN PROKARYOTES AND EUKARYOTES

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Contents

Summary

An amazing diversity in metabolic strategies is found in Nature. This diversity is expressed both at the level of dissimilatory metabolism (energy-generating strategies) and assimilatory metabolism (the way new cell material is built). The greatest variety in

ways to make a living is found among the prokaryotes (*Bacteria* and *Archaea*). Energy can be obtained using light as an energy source (phototrophic organisms) or by using chemical transformations. In the latter case, organic compounds most often provide energy (chemoorganotrophic metabolism). There are also bacteria that use reduced inorganic compounds as an energy source (chemoautotrophic metabolism). All three types of energy generation may occur both in the presence and in the absence of molecular oxygen. With respect to the carbon source used for assimilatory purposes, heterotrophy (the use of organic carbon sources) is opposed to autotrophy (use of carbon dioxide as carbon source). Autotrophy can be coupled both to the use of light and to the use of reduced inorganic compounds as the energy source. The biogeochemical cycles of elements such as carbon, nitrogen, sulfur, iron, and others function thanks to the metabolic diversity among microorganisms, especially as far as dissimilatory metabolism is concerned. Microorganisms catalyze the chemical conversions, especially those involving changes in the oxidation state of the elements involved. It is the metabolic diversity that supports the recycling of matter and the functioning of all ecosystems on Earth.

1. Introduction

The functioning of the biogeochemical cycles of carbon, nitrogen, sulfur, and other elements on Earth depends to a large extent on the metabolic diversity of microorganisms, prokaryotic as well as eukaryotic. Especially in the prokaryote world, the variety of ways to produce energy is amazing, and many of the dissimilatory (energy-yielding) processes performed by bacteria play important roles in the functioning of ecosystems, from small-scale processes to the global level. Not only are organic compounds continuously being synthesized and degraded, but important transformations of inorganic compounds also occur. Thus, ammonium ions are oxidized to nitrate by nitrifying bacteria, biologically available nitrate may be lost in the form of the biologically much more inert molecular nitrogen during the process of denitrification, and sulfate is reduced to sulfide in the absence of oxygen. Such oxidation and reduction reactions are an integral part of the cycle of matter of Earth.

This chapter presents an overview of the metabolic diversity among microorganisms, with special emphasis on their dissimilatory processes. The dissimilatory part of cell metabolism in which energy is generated is most relevant here, as firstly, the diversity here is much more pronounced than in the case of the assimilatory processes, and secondly, the amount of matter transformed by microorganisms to provide energy for growth greatly exceeds the amount of material taken up for assimilatory purposes. Metabolic diversity in the prokaryote world (*Archaea* as well as *Bacteria*) is much greater than that displayed by the eukaryotes (*Eucarya*). Moreover, the metabolism of the bacteria is generally much faster and their generation times are much shorter, potentially leading to very rapid turnover of matter. Therefore, this chapter will primary deal with processes driven by prokaryotes.

2. The Thermodynamic and Mechanistic Basis of Cellular Metabolism

A proper insight in to the diversity displayed by microorganisms in their dissimilatory and assimilatory metabolism can only be obtained within the framework of a basic

understanding of the bioenergetic processes underlying cell metabolism. All living organisms require energy in order to perform the processes inherent to life, such as growth and multiplication, movement, reaction to stimuli from their environment, etc. The sources of energy used in the living world can be divided into two groups: physical (light) and chemical.

The amount of energy available to drive useful work (Gibbs free energy) released in the course of the dissimilatory process performed is the most important bioenergetic parameter for any living organism. Therefore the change in free energy (ΔG) of any reaction or process determines whether it can (at least theoretically) be used for energy generation. When the change in free energy is positive, i.e., the amount of free energy in the reaction products exceeds the amount of free energy in the reagents, the reaction is called endergonic; when the total free energy in the products is lower than in the reagents, the reaction is exergonic. In the latter case, free energy is released, and this energy can be used to drive energy-requiring biochemical processes.

The change in free energy during any chemical reaction can be calculated according to:

$$\Delta G^{o} = \sum \Delta G_{f}^{o}(\text{products}) - \sum \Delta G_{f}^{o}(\text{reagents}) \qquad (1)$$

in which ΔG_f^o represents the free energy (in kJ mol^{-1}) required for the synthesis of the compounds involved from the elements of which they are composed. The sign "^{o}" signifies that all calculations relate to standard conditions: all compounds that participate in the reaction are present at a concentration of 1 M (or in case of gases: 1 atmosphere), and the reaction temperature is 25°C. Table 1 presents values of G_f^o for a number of key compounds relevant to the energy metabolism of prokaryotic and eukaryotic microorganisms.

Compound	**Free Energy KJ kJ mol^{-1}**		**Compound**	**Energy Kj kj mol^{-1}**
CO_2	- 394.4		S^o	0
HCO_3^-	- 586.9		SO_3^{2-}	- 486.6
CO	- 137.3		SO_4^{2-}	- 744.6
CH_4	- 50.8		$S_2O_3^{2-}$	- 513.4
$Acetate^-$	- 369.4		H_2S	- 27.9
$Butyrate^-$	- 352.6		HS^-	+ 12.1
Methanol	- 175.4		S^{2-}	+ 85.8
Ethanol	- 181.8			
$Formate^-$	- 351.0		O_2	0
$Fumarate^{2-}$	- 604.2			
Glucose	- 917.2		N_2	0
$Lactate^-$	- 517.8		NO	+ 86.6
$Propionate^-$	- 361.1		NO_2^-	- 37.2
$Succinate^{-2}$	-690.2		NO_3^-	- 111.3

			NH_4^+	- 79.4
Fe^{3+}	- 4.6		N_2O	+ 104.2
Fe^{2+}	- 78.9			
FeS_2	- 166.9			
H_2O	- 237.2			
H^+	0 at pH 0 (- 5.69 per pH unit; - 39.8 at pH 7)		OH^-	- 157.3 at pH 14; - 198.8 at pH 7

Table 1. Free energy of formation from the elements (G_f^o) of several compounds of biological interest (in kJ mol^{-1}).

In the case of biological reactions, it is customary to calculate the thermodynamic parameters at pH 7 and not at pH 0 (= a proton concentration of 1 M), and then the change in free energy is notated as $\Delta G^{o\prime}$. The relationship between $\Delta G^{o\prime}$ and ΔG^o is:

$$\Delta G^{o\prime} = \Delta G^o + m\Delta G_f^{\prime}(H^+) \qquad (2)$$

in which *m* is the number of protons involved in the reaction (*m* is negative when protons are consumed), and $\Delta G^{\prime}_f$ (H^+) represents the free energy of formation of a proton (39.8 kJ mol^{-1} at pH 7).

With the help of data such as those appearing in Table 1, it is possible to calculate the change in free energy ($\Delta G^{o\prime}$) that accompanies chemical reactions, such as those that occur in dissimilatory and assimilatory cell metabolism. For example, the free energy change that accompanies the fermentation of glucose to two molecules of lactic acid (Glucose $\rightarrow$ 2 $Lactate^-$ + 2 H^+) can be quantified as 2 × (- 517.8) + 2 × (- 39.8) - (- 917.2) = - 198 kJ.

It should be noted that the true change in free energy does not depend on the $\Delta G^{o\prime}$ of the reaction only, but also on the actual concentrations of the reagents and the products. In a reaction that can be written as:

$$aA + bB \rightarrow cC + dD \qquad (3)$$

in which A-D are the compounds participating in the reaction and a-d are the stoichiometric ratios of the molecules of each compound, the relation between $\Delta G^{\prime}$ (the actual change in free energy at pH 7) and $\Delta G^{o\prime}$ is:

$$\Delta G^{\prime} = \Delta G^{o\prime} + RT\ln + \frac{[C]^c \cdot [D]^d}{[A]^a \cdot [B]^b} \qquad (4)$$

a formula in which [A] represents the concentration of compound A, etc., T is the temperature in °K, and R is the gas constant (8.29 J mol^{-1} $^{o}K^{-1}$).

Many groups of microorganisms obtain their energy from redox reactions (for example: processes of aerobic respiration, anaerobic respiration, oxidation of reduced inorganic compounds). In these reactions, the change in free energy under standard conditions is proportional to the difference in the standard redox potential between the electron acceptor and the electron donor involved according to:

$$\Delta G^{o\prime} = -nF\Delta E_o^{\prime} \qquad (5)$$

in which n represents the number of electrons transferred in the reaction, F is the Faraday constant (96.5 kJ V^{-1}), and $E_o^{\prime}$ represents the difference in reduction potential of the redox couples participating in the reaction. Table 2 presents the $E_o^{\prime}$ values of a number of redox couples of biological importance.

The more reduced the electron donor and the more oxidized the electron acceptor is, the more energy can be gained from the reaction. Therefore molecular oxygen ($E_o^{\prime}$ of the couple O_2/H_2O = + 0.82 V) is the preferred electron acceptor, and the maximal amount of free energy can be gained during oxidation of organic compounds when using oxygen as the terminal electron acceptor, for example:

$$\text{Glucose} + 6\,O_2 \rightarrow 6\,CO_2 + 6\,H_2O \qquad \Delta G^{o\prime} = -2872\ \text{kJ} \qquad (6)$$

Redox pair	$E_o^{\prime}$ (V)
H^+/H_2	- 0.41
$NAD^+/NADH$	- 0.32
S^o/HS^-	- 0.27
CO_2/CH_4	- 0.24
Acetaldehyde/Ethanol	- 0.20
$Pyruvate^-/Lactate^-$	- 0.19
HSO_3^-/HS^-	- 0.12
$APS/AMP + HSO_3^-$	- 0.06
$Fumarate^{2-}/Succinate^{2-}$	+ 0.03
NO_2^-/NO	+ 0.35
NO_2^-/NH_4^+	+ 0.44
NO_3^-/NO_2^-	+ 0.43
Fe^{3+} / Fe^{2+}	+ 0.77
O_2 /H_2O	+ 0.82
NO/N_2O	+ 1.18
N_2O/N_2	+ 1.36

Table 2. Standard reduction potential of a number of redox pairs of biological importance ($H^+/H_2 = 0$ at pH = 0). APS = adenosine-5´-phosphosulfate, the activated form of sulfate.

This energy is sufficient for the formation of 38 molecules of ATP. The formation of ATP from ADP and inorganic phosphate costs about 31.8 kJ mol^{-1} under standard conditions, but under the non-equilibrium conditions of the living cell, the true amount of free energy needed to drive the formation of ATP is close to 70 kJ mol^{-1}. This value both takes into account the true concentrations of ATP, ADP and phosphate within the cell and the inevitable amount of energy lost as heat during the process.

In every living cell, there are two forms of energy available to the cell metabolism: (1), chemical energy in the form of ATP and a number of additional compounds possessing high-energy bonds, and (2), the electrochemical gradient of protons (also termed proton motive force, $\Delta\mu_H^+$), in rare cases accompanied by gradients of other ions such as Na^+, between the two sides of the cell membrane of prokaryotes, the inner membrane of mitochondria, or the thylakoids of chloroplasts. These two forms of energy are interchangeable by means of action of the ATP synthase located in the membrane. This enzyme enables the formation of ATP at the expense of the proton electrochemical gradient, and when acting in the opposite direction, it enables the build-up of the $\Delta\mu_H^+$ at the expense of high-energy bonds in ATP. In some of the modes in which organisms generate energy, ATP formation is the primary process (substrate-level phosphorylation, e.g., during bacterial and yeast fermentation). In other types of metabolism, such as electron transport during respiration or photosynthesis, the formation of the proton electrochemical gradient is the primary process, and ATP is produced as a secondary process in the course of the dissipation of the proton gradient ("electron transport phosphorylation").

Substrate level phosphorylation is based on the formation of compounds possessing phosphate groups bound with high-energy bonds (in most cases phosphate anhydride bonds), generated during the breakdown of organic compounds. Enzymatic reactions enable the substitution of this high-energy bond for another high-energy bond: the bond between the terminal phosphate and ADP in the ATP molecule. The number of different high-energy compounds used by the cell to produce ATP is limited. Table 3 presents the most important high-energy molecules involved in substrate-level phosphorylation.

High-energy compound	**$\Delta G^{o\prime}$ of hydrolysis (kJ mol^{-1})**
1,3-Bisphosphoglycerate	- 51.9
Phosphoenolpyruvate	- 51.6
Acetyl phosphate	- 44.8
Acetyl-CoA	- 35.7
Carbamyl phosphate	- 39.3
Adenosine-5'-phosphosulfate	- 88.0
Succinyl-CoA	- 35.1

Table 3. The most common high-energy compounds involved in

substrate-level phosphorylation.

The second mode of energy generation involves the transfer of electrons through a chain of electron carriers such as flavins, quinones, and cytochromes located in membranes. Within the chain of redox reactions in which electrons are transferred from carrier to carrier, certain stages exist in which protons (H^+) are consumed, and others in which protons are liberated. The components of the electron transport chain are so arranged in the membrane that protons are consumed at the inner side (cytoplasmatic, inner thylakoid space) of the membrane, and are liberated at the opposite side. Thus an electrochemical gradient of protons is formed, that will manifest itself as a difference in pH (alkaline inside) and an electrical potential (negative inside). In rare cases, sodium ions (Na^+) may replace the protons, and the result is a primary gradient of sodium ions. With the help of the ATP synthase located in the membrane, the electrochemical gradient of protons across the membrane can be transformed into ATP.

We find electron transport chains in biological membranes both in respiration processes and in photosynthesis. During respiration, a reduced compound (generally organic, in special cases also inorganic) molecule is oxidized. The electrons released are transferred through carriers in the cell membrane to a terminal electron acceptor. The final electron acceptors in respiration are oxygen (in the case of aerobic organisms) or other oxidized inorganic compounds, such as nitrate (NO_3^-) or sulfate (SO_4^{2-}) (a process termed anaerobic respiration). When electron flow is from a low redox potential to a high one (see Table 2), the process is exergonic, and part of the free energy released is conserved in the form of a proton electrochemical gradient. Photosynthesis is based on the use of light as the energy source. As a result of the absorption of photons by chlorophyll or bacteriochlorophyll molecules in the photosynthetic reaction centers, low-potential electrons are formed, and these are transferred though a chain of electron carriers in the photosynthetic membranes under formation of a primary proton gradient.

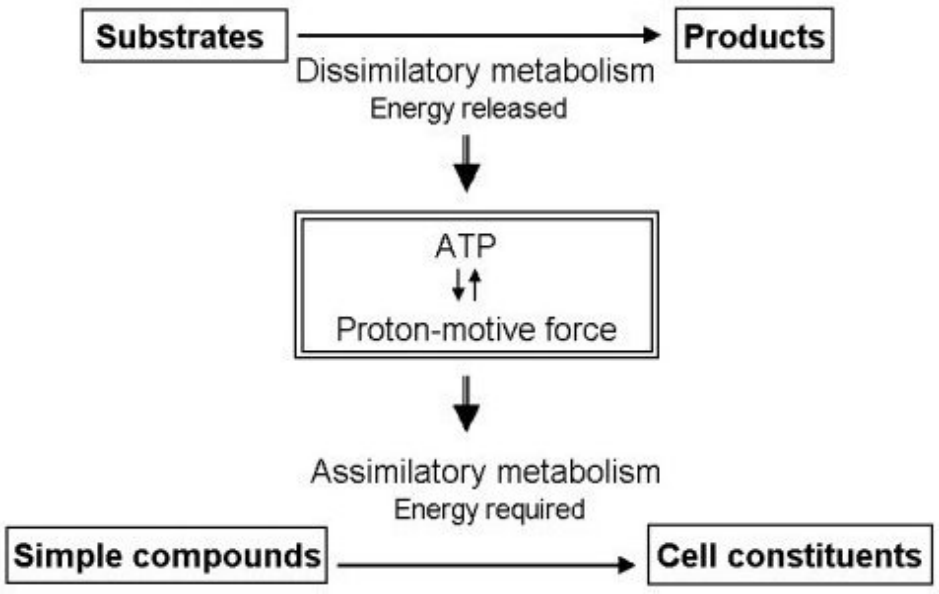

Figure 1. The function of ATP and the primary electrochemical gradient of protons ($\Delta\mu_H^+$) as a mediator between assimilatory and dissimilatory metabolism.

All processes mentioned above supply energy for the existence of the cell (dissimilatory processes). The energy that the cell gains in the form of ATP and/or in the form of a primary electrochemical gradient of protons is utilized for all endergonic processes in the cell, such as assimilation of nutrients, biosynthesis of proteins and other macromolecules (assimilatory processes), and also for maintenance purposes. The pool of available energy in the form of ATP and/or $\Delta\mu_H^+$ is the mediator between the dissimilatory and the assimilatory processes (Figure 1).

2.1. Dissimilatory Metabolism

There are two basically different modes of obtaining energy in the living world: by the use of light energy (the phototrophic way of life) and by the use of chemical energy (the chemotrophic way of life). Chemotrophs can then be further divided into (chemo)heterotrophs or (chemo)organotrophs - organisms that degrade organic compounds as their source of energy, and chemoautotrophs or chemolithotrophs - organisms that oxidize reduced inorganic compounds as their energy source (Figure 2). In the sections below, the basic nature of these processes is explained, and key examples of each type of organism are discussed.

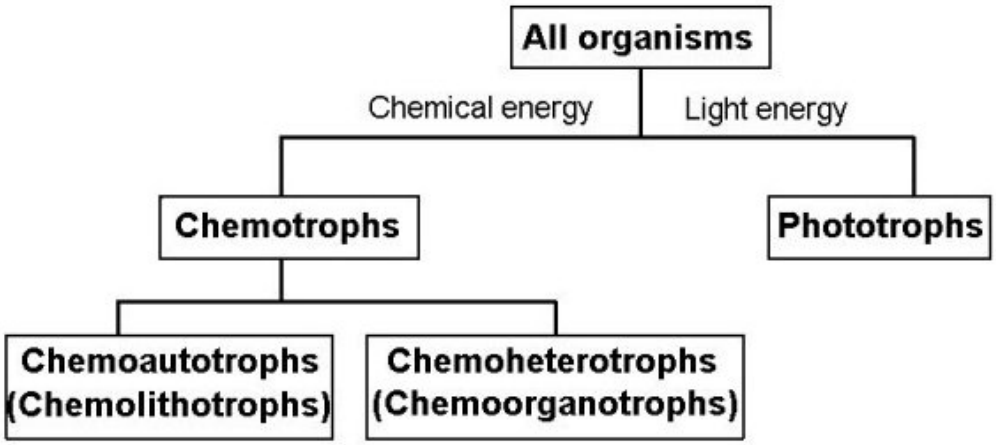

Figure 2. Classification of the living organisms according to their mode of energy generation.

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Biographical Sketch

Prof. Aharon Oren was born in 1952 in Zwolle, the Netherlands. He obtained his M.Sc. degree (microbiology and biochemistry) (1974) from the University of Groningen and his Ph.D. degree in microbiology (1978) from the Hebrew University of Jerusalem. He has been on the staff of the Hebrew University as research fellow (1979-1982), lecturer (1984-1985), senior lecturer (1985-1991), associate professor (1991-1996) and professor of microbial ecology (since 1996). He has been post-doctoral fellow (1982-1983) and visiting assistant professor (1983-1984) at the University of Illinois at Urbana-Champaign. Functions performed by Prof. Oren include: member (since 1989) and secretary (since 1994) of the International Committee on Systematics of Prokaryotes subcommittee on taxonomy of Halobacteriaceae, member of the International Committee on Systematics of Prokaryotes subcommittee on the taxonomy of photosynthetic prokaryotes (since 1991), member of the editorial board of the International Journal of Salt Lake Research (1991-1999), editor of FEMS Microbiology Letters (1994-present), director of the Moshe Shilo Minerva Center for Marine Biogeochemistry (1995-2000), head of the Division of Microbial and Molecular Ecology, The Institute of Life Sciences at the Hebrew University of Jerusalem (1998-2003), co-opted member (1999-2002), chairman (2002-2008), and executive secretary/treasurer (2008-present) of the International Committee on Systematics of Prokaryotes, member of the Executive Committee (1999-2002) and vice-president (2002-present) of the International Society for Salt Lake Research affiliate professor of George Mason University, Fairfax, Virginia (1999-present), associate member of Bergey's Manual Trust (2001-present), member of the editorial board of Extremophiles (2001-present), associate editor of the International Journal of Systematic and Evolutionary Microbiology (2001-present), member of the Editorial Review Board of Archaea (2004-present), and associate editor of Saline Systems (2005 – present). Prof. Oren was the recipient of the Moshe Shilo prize of the Israel Society for Microbiology for 1993 and of the Ulitzki prize of the Israel Society for Microbiology for 2004, and was elected fellow of the American Academy of Microbiology in 2000. Prof. Oren has published nine books and over three hundred research articles, reviews, and book chapters.