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Review

Bioenergetics and anaerobic respiratory chains of aceticlastic methanogens ☆

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ABSTRACT

Methane-forming archaea are strictly anaerobic microbes and are essential for global carbon fluxes since they perform the terminal step in breakdown of organic matter in the absence of oxygen. Major part of methane produced in nature derives from the methyl group of acetate. Only members of the genera *Methanosarcina* and *Methanosaeta* are able to use this substrate for methane formation and growth. Since the free energy change coupled to methanogenesis from acetate is only −36 kJ/mol CH_4, aceticlastic methanogens developed efficient energy-conserving systems to handle this thermodynamic limitation. The membrane bound electron transport system of aceticlastic methanogens is a complex branched respiratory chain that can accept electrons from hydrogen, reduced coenzyme F_{420} or reduced ferredoxin. The terminal electron acceptor of this anaerobic respiration is a mixed disulfide composed of coenzyme M and coenzyme B. Reduced ferredoxin has an important function under aceticlastic growth conditions and novel and well-established membrane complexes oxidizing ferredoxin will be discussed in depth. Membrane bound electron transport is connected to energy conservation by proton or sodium ion translocating enzymes ($F_{420}H_2$ dehydrogenase, Rnf complex, Ech hydrogenase, methanophenazine-reducing hydrogenase and heterodisulfide reductase). The resulting electrochemical ion gradient constitutes the driving force for adenosine triphosphate synthesis. Methanogenesis, electron transport, and the structure of key enzymes are discussed in this review leading to a concept of how aceticlastic methanogens make a living. This article is part of a Special Issue entitled: 18th European Bioenergetic Conference.

1. Introduction

Methane producing microorganisms belong to the domain of the *Archaea* and are of great interest because of their important ecological function and their unique biochemical features. Methanogens are strictly anaerobic organisms and are found in anoxic environments such as fresh water sediments, tundra areas, swamps and the intestinal tract of ruminants and termites as well as in man-made environments such as rice fields, anaerobic digesters of sewage plants and biogas plants [1,2]. The formation of methane belongs to the most important global bioelement fluxes because it marks the end of the anaerobic food chain for the recycling of carbon components from organic matter [3]. In this process biopolymers are hydrolyzed to mainly sugars, amino acids, purines, pyrimidines, fatty acids and glycerol. Fermentative bacteria convert these organic compounds to simple carbonic acids (e.g. propionate, butyrate and acetate), alcohols (e.g. ethanol, propanol and butanol) and some other compounds (e.g. H_2, CO_2 and ketones). These products are used as substrates by syntrophic bacteria, which form acetate and $H_2 + CO_2$ [4–6]. These end products of fermentative degradation are then converted to methane by methanogenic archaea [7]. The major part of the methane released from anaerobic habitats is oxidized by aerobic bacteria. However, billions of metric tons of CH_4 per year escape into the atmosphere where methane contributes to global warming since it is a 20 times more potent greenhouse gas than carbon dioxide [8,9]. In addition, methanogens are involved in the formation of the so-called methane hydrates [10] which may represent one of the largest sources of hydrocarbon on earth [11]. These methane-trapping, water-ice-like structures are naturally formed at high pressures and low temperatures, and are found within ocean continental slopes and in permafrost regions. There are indications that climatic changes in the past were based on large releases of methane into the atmosphere. Therefore, concerns have arisen about the possible impacts of a temperature increase on the present deposits of methane hydrates [12]. On the other hand methanogens are an integral part of biogas reactors and essential for the production of the combustible gas methane that is a renewable energy source, and is used for the generation of electricity and heat. In Germany alone more than 7000 biogas plant were built until 2011 producing about 3000 MW of electric power, a capacity that is comparable to the performance of about three atomic power plants [13]. Moreover, methane-enriched and purified biogas can be fed into gas distribution

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networks and can replace natural gas as a feedstock for producing chemicals and materials. Hence, biogas production has a huge economical value and is of interest in order to substitute fossil fuels.

2. Acetate converting methanogens

Methanogenic archaea are divided into seven taxonomic orders each of which is as distantly related to the other as humans are to slime moulds. Species of the orders Methanobacteriales, Methanococcales, Methanomicrobiales, Methanocellales, Methanopyrales and Methanoplasmatales are obligate hydrogenotrophic organisms and use $H_2 + CO_2$ (or in some cases H_2 + methanol) as substrate [14]. In addition, most of them can oxidize formate that is converted to $H_2 + CO_2$ and then used for methanogenesis. However, the obligate hydrogenotrophic methanogens are not able to utilize acetate [15] and are therefore not discussed in this review (for a detailed description of these methanogens see [9,16–19]). The major part of methane in nature derives from the methyl group of acetate but only two genera, namely *Methanosarcina* and *Methanosaeta*, have been described to use this substrate for methanogenesis and growth [20].

Members of the genus *Methanosarcina* have the broadest substrate spectrum of all methanogens and use acetate, methanol and other methylated C_1 compounds such as methylamines and methylated thiols for methane formation. Some of them are also able to utilize $H_2 + CO_2$ [21]. The species reveal a number of distinct morphological forms including single cells and sarcina packages, as well as multicellular packets and lamina [22]. Single cells are coccoid and are covered by S-layer proteins that are sometimes overlaid by sheets of heteropolysaccharides. This structure is called methanochondroitin and closely resembles eukaryotic chondroitin [22]. Furthermore, *Methanosarcina* species are unsurpassed among methanogens in terms of metabolic, physiological and environmental versatility. These capabilities are reflected in the genomes of *Methanosarcina* (*Ms.*) *acetivorans* (5.7 Mbp), *Ms. barkeri* (4.8 Mbp) and *Ms. mazei* (4.1 Mbp) that are more than twice as large as the genomes of obligate hydrogenotrophic methanogens [23–25]. The genomes reveal extensive genetic diversity and redundancy underlining the ability of *Methanosarcina* strains to adapt to various environmental conditions. In addition, the genomes indicate the potential for entirely unexpected metabolic capabilities. One of the most interesting features is the fact that *Methanosarcina* species obviously acquired hundreds of eubacterial genes [26], among them many which encode subunits of key enzymes of the energy conserving machinery.

Species of the genus *Methanosaeta* form rod-shaped cells and are normally combined end to end in long filaments, surrounded by a sheath-like structure [27]. While *Methanosarcina* species are metabolically versatile, members of the genus *Methanosaeta* are specialized on acetate degradation. A minimal concentration of only 7–70 μM is needed for growth indicating a high affinity to this substrate [28,29]. From the genome sequences of *Methanosaeta* (*Mt.*) *thermophila* (1.9 Mbp) [30], *Mt. harundinacea* (2.6 Mbp) [31] and *Mt. concilii* (3.0 Mbp) [32] and from pathway reconstruction [30,33] it is evident that the key enzymes of the core processes of methane formation from acetate are similar to the ones found in *Methanosarcina* species.

3. Biochemistry of methanogenesis

In this review we will focus on methane formation from acetate and will only briefly present data on methanogenesis from other substrates. For further reading we refer to excellent reviews that have been published in the last five years [9,16,20,34–36].

3.1. Methanogenic cofactors

Several unusual coenzymes and prosthetic groups participate in methanogenesis of aceticlastic methanogens [37], which are referred to as methanofuran (MFR), tetrahydrosarcinapterin (H_4SPT), 2-mercaptoethanesulfonate (coenzyme M or HS-CoM), *N*-7-mercaptoheptanoyl-L-threonine phosphate (coenzyme B or HS-CoB), coenzyme F_{420} (F_{420}), coenzyme F_{430} and methanophenazine (Mph) (Fig. 1). MFR und H_4SPT are carriers of C_1-fragments between formyl and methyl oxidation levels in the process of methanogenesis from $H_2 + CO_2$ and methylated C_1 compounds. HS-CoM is a ubiquitous methyl group carrier in the pathway of methanogenesis. The methylated form, CH_3-S-CoM, is the substrate for the methyl-CoM reductase that catalyzes the terminal step of methanogenesis. This enzyme contains another unusual and unique methanogenic cofactor called coenzyme F_{430} [38–40]. It is made of a reduced tetrapyrrole ring system that coordinates a nickel ion [41,42]. HS-CoM, F_{420} and Mph function as electron carriers in methanogenesis (Fig. 1) [19,37]. F_{420} is a deazaflavine derivative with a midpoint potential of −360 mV and is a central electron carrier in the cytoplasm of methanogens [43]. HS-CoB is used as electron donor in the process of methane formation from CH_3-S-CoM as catalyzed by the methyl-CoM reductase (Fig. 2) [44,45]. The last unusual cofactor discovered in *Methanosarcina* species was Mph (Fig. 1). It functions as electron carrier within the cytoplasmic membrane with a mid-point potential of −165 mV that replaces quinones that are not present in methanogens. This cofactor represents a 2-hydroxyphenazine derivative that is linked via an ether bridge to a pentaisoprenoid side chain [46–48].

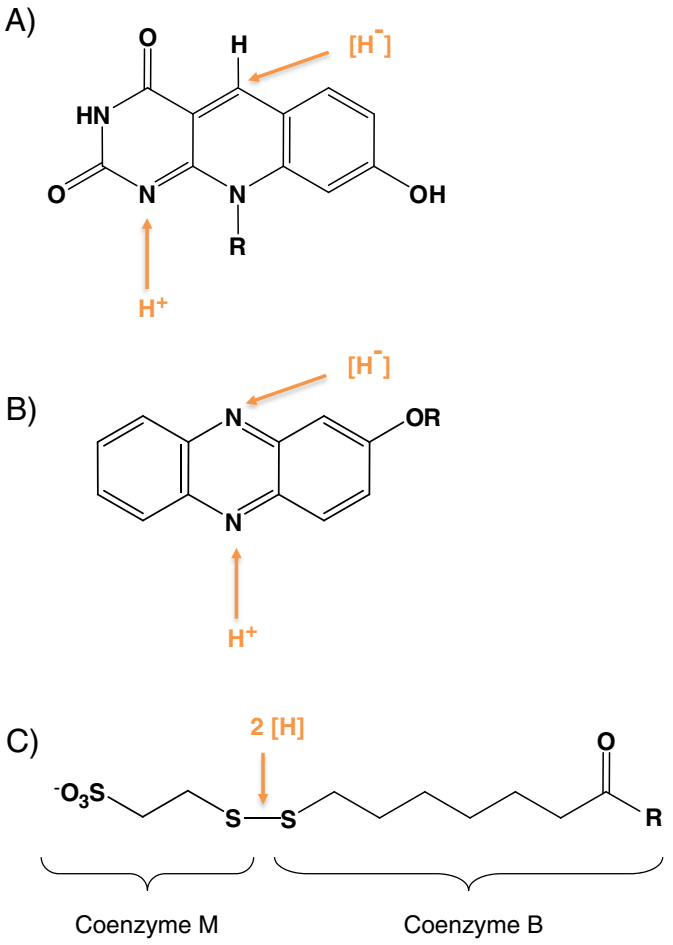

Fig. 1. Chemical structure of electron carriers involved in methanogenesis. (A) F_{420}, (B) Mph, (C) CoM-S-S-CoB (the parts of the molecule representing coenzyme M and B are indicated). Only the oxidized form of the reactive part of the cofactors is shown. The positions for reduction are indicated in red.

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