THEORETICAL AND EVOLUTIONARY BIOLOGY

Chemical Basis of Carbon Fixation Autotrophic Paleometabolism

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Received November 17, 2019; revised March 4, 2021; accepted March 4, 2021

Abstract—On the basis of biomimetic, phylometabolic, and thermodynamic analysis of modern CO₂ assimilation pathways, a paleophenotypic reconstruction of ancient autotrophic metabolism systems was carried out. As a chemical basis for CO₂ fixation paleometabolism, metabolic networks capable of self-reproduction and evolution are considered, and the reversibility of the transformation reactions of its intermediates is the most important factor in self-development of this network. The substances of the C–H–O system, paragenetically associated with hydrocarbons, create a phase space, which is a set of universal intermediates of the autotrophic paleometabolism chemical network. The concept of two strategies for the origin and development of autotrophic carbon fixation paleometabolism in the oxidized (CO₂) and reduced (CH₄) redox regimes of degassing of the ancient Earth is proposed. It was shown that P, T, and the redox conditions of hydrothermal systems of the early Archean were favorable for the development of primary methanotrophic metabolism.

Keywords: chemoautotrophic fixation of CH₄ and CO₂, biomimetic models, universal intermediates of metabolism, chemical reaction network, hydrocarbons, hydrothermal systems of the early Archean, primary methanotrophy

DOI: 10.1134/S1062359021050095

INTRODUCTION

Paleometabolism is the metabolism of phylogenetically identified evolutionarily ancient organisms, as well as the metabolism that existed in the extinct ancestors of modern organisms. The fixation of inorganic carbon into organic material (autotrophy) is a prerequisite for life and the starting point of biological evolution, and paleometabolism of autotrophic carbon assimilation was obviously the source of biomass and the basis for the functioning of the first protocells on ancient Earth.

In the modern autotrophic metabolism creating bioorganic substances, carbon is assimilated mainly in the form of carbon dioxide, and the existing biochemical CO₂ fixing pathways are the foundation of biomimetic modeling of autotrophic paleometabolism on the ancient Earth. However, there is evidence that the atmosphere and hydrosphere of the early Archean was enriched in hydrogen and methane (Tian et al., 2005; Catling and Kasting, 2017; Zahnle et al., 2019), and the continental crust of the early Archean was more reduced than the modern one (Yang et al., 2014).

The reductive hydrothermal environment on ancient Earth implies the possibility that the first autotrophic metabolic systems were able to use methane as the main source of carbon, i.e., possess methane-fixation paleometabolism, which was subse-

quently lost in the course of evolution or thrown into extreme ecological niches. In this work, based on biomimetic, phylometabolic, and thermodynamic analyses of carbon-fixation pathways, the chemical models of prebiotic fixation systems for CO₂ and CH₄, which were the chemical basis of nascent chemoautotrophic paleometabolism, are considered.

CO₂ fixation paleometabolism. Nature uses alternative pathways for carbon fixation and currently six different autotrophic CO₂-fixation pathways have been found: one linear (Wood–Ljungdahl (WL) reductive pathway (methanogenic and acetogenic acetyl–CoA pathways in archaea and bacteria, respectively) and five cyclic. These include the tricarboxylic acid (TCA) cycle (reductive citrate cycle, Arnon–Buchanan cycle), the 3-hydroxypropionate (3-HP) bicycle, the reductive dicarboxylate/4-hydroxybutyrate cycle, the 3-hydroxypropionate/4-hydroxybutyrate cycle, and the reductive pentose–phosphate (PP) (Calvin–Benson–Bassam) cycle. All six known pathways of CO₂ fixation are the final evolutionary branches of the ancestral paleometabolic core of the last universal common ancestor (LUCA), which is an implied evolutionary intermediate linking the abiotic phase of Earth's history with the first traces of microbial life (biological activity, stromatolites, and microfossils) found in rocks with an age of 3.7 Ga (Nutman et al.,

2016). However, LUCA is already an incredibly complex structure surrounded by a membrane with developed enzyme-driven systems of replication and metabolism (Martin et al., 2016; Weiss et al., 2018), which emerged as a result of the evolution of chemical system C–H–O in hydrothermal mineral systems.

The modern TCA cycle, as one of the most evolutionarily ancient ways of CO₂ fixation, was proposed and substantiated as a model of the primary anabolic chemical system of CO₂ fixation (Wächtershäuser, 1990, 1992; Smith and Morowitz, 2004; Hügler and Sievert, 2011). At the same time, a number of arguments indicate the primacy of the aceto- and methanogenic WL pathway of CO₂ fixation (Russell and Martin, 2004; Martin and Russell, 2007; Weiss et al., 2016; etc.) and the 3-HP bicycle of CO₂ fixation (Marakushev, 2008; Marakushev and Belonogova, 2013b).

The emergence of the functionality of autonomous chemical functional systems is obviously at the heart of biological evolution (Ruiz–Mirazo et al., 2017). The formation of “self-sustaining autocatalytic networks” as the chemical basis of the emerging paleometabolism of LUCA has been shown theoretically in many studies (e.g., Hordijk and Steel, 2018), and the boundary modular design of the intermediates of CO₂ fixation cycles allowed to create models of protometabolic systems in the form of a chemical network of symbiosis of specific pathways (Marakushev, 2008; Marakushev and Belonogova, 2010, 2013a, 2013b; Braakman and Smith, 2012, 2013; Marakushev and Belonogova, 2013). In these models, the branching points (bifurcation nodes) determine the development of the chemical network in different directions, depending on changes in the physicochemical conditions of the environment. Thermodynamic calculations have shown the possibility of functioning of autotrophic cycles in the forward and reverse directions (Marakushev and Belonogova, 2009, 2012, 2013a, 2013b), i.e. the possibility of the initiation of oxidative or reductive cycles, leading to the appearance of autotrophic, heterotrophic and mixotrophic metabolism. A phylometabolic comparison of the metabolic cores of deeply rooted microorganisms with related organisms both within and between adjacent branches also led to a model of a primary modular combinatorial CO₂ fixation network (Braakman and Smith, 2012, 2013).

The minimal combinatorial chemical network of autotrophic protometabolism can be represented as a combination of the WL pathway, the TCA cycle, and the 3-HP bicycle (Fig. 1). Their coupling is carried out in the nodal reactions of changing the electron flow direction. The succinate ↔ fumarate reaction binds TCA and 3-HP cycles, while the acetate ↔ pyruvate reaction adds to this network the WL pathway, which consists of two branches of hydrogenotrophic (Ia) and methanotrophic (Ib) acetogenesis. Acetate carboxylation completes the WL path: CH₃COOH (acetate) +

CO₂ + H₂ = CH₃(CO)COOH (pyruvate) + H₂O or initiates a 3-HP bicycle: CH₃COOH (acetate) + CO₂=CH₂(COOH)₂ (malonate).

Chemical reactions (Table 1) with negative free energy (exergonic) release energy as they occur. Obviously, the reversibility of reactions is a key factor in the functioning and evolution of this network. For example, the free energy of the key disproportionation reaction citrate → oxaloacetate + acetate fluctuates around zero at both physiological temperatures (–7.24 kJ) and at 473 K (–15.15 kJ) (Table 1), and recent biochemical studies have shown the complete reversibility of the citrate cycle in *Nitrospirae*, which is largely determined by this reversible “ligase” reaction (Nunoura et al., 2018; Mall et al., 2018). The succinate ↔ fumarate reaction is a redox switch for the direction of electron flow between 3-HP and TCA cycles; i.e., the value of the free energy of the reaction succinate ↔ fumarate ($G_{298}^0 = \pm 102.24$ kJ) (Table 1) can be conventionally considered a certain criterion and the limit of reversibility of the reactions of all protometabolic cycles. Such thermodynamic control is realized by a multistep of reactions with a small change in the Gibbs free energy, and this is an important factor in the reversibility of the supercycle modules functioning. Therefore, phosphoenolpyruvate could no longer become an intermediate of this metabolic network, since the phosphorylation reactions of pyruvate and oxaloacetate are practically irreversible (Table 1), which is evidence in favor of the hypotheses of primary phosphorus-free metabolism (Goldford et al., 2017).

Based on a simple set of structural constraints obtained from the physical and chemical considerations, a set of intermediates of 153 organic substances (40 compositions) of the C–H–O system was determined for theoretical calculations of all possible combinations of intermediates of the CO₂ fixation cycles (Morowitz et al., 2000). Computer simulations using the thermodynamic and kinetic characteristics of the reactions of carboxylation, hydration, hydrogenation, and cleavage show that the “space” of the chemical structures of intermediates with optimal characteristics for CO₂ fixation is several times larger than the set of Morowitz intermediates (Meringer and Cleaves, 2017). The entire set of these substances can be represented on the phase compositions diagram (Fig. 2). The C–H₂O equilibrium divides the diagram into oxidative (I) and reductive (II) facies. All compositions of the Morowitz and Meringer substances are located in the oxidative facies I. The set of intermediates for the common metabolic core can be expanded significantly by the involvement of hydrocarbons in the protometabolic network (Zubarev et al., 2015). In this case, the “chemical space” of the intermediates is significantly shifted to the reductive facies II (Fig. 2). It is important that all intermediates of the universal core of intermediate metabolism are located within this space (Morowitz et al., 2000; Marakushev and Belo-

Fig. 1. The chemical network of protometabolism, consisting of three biomimetically reconstructed pathways of CO_2 fixation with possible collective autocatalysis, as a developing capable functional core. Disproportionation reactions of malate, citrate, and citramalate are shown by dash–dotted lines. Biomimetic pathways of CO_2 fixation: Ia, hydrogenotrophic and, Ib, methanotrophic acetogenesis; II, citrate cycle; III, 3-hydroxypropionate bicycle. There are shown reversible reactions revealed in biochemistry (Mall et al., 2018) and model experiments (Muchowska et al., 2017, 2019; Ralser, 2018; Varma et al., 2018). Irreversible reactions of phosphorylation of pyruvate and oxaloacetate initiate the PP cycle of CO_2 fixation (formation of phosphoenolpyruvate).

nogova, 2009, 2010; Braakman and Smith, 2012, 2013; Goldford et al., 2017). On the C–H–O composition diagram (Fig. 2), they are located inside the triangle with the CH_4 – C_2H_4 hydrocarbon base. Hydrocarbons are often found in gas–liquid inclusions of deeply generated minerals (Potter and Konnerup-Madsen, 2003), and a recent remarkable discovery is the detection in Archean quartz from the Australian Jack Hills conglomerates the inclusions of abiogenic hydrocarbons and simple organic substances (Schreiber et al., 2017), which in those days could have been carbon sources for the emerging metabolism. On the composition diagram (Fig. 2), they are mostly located in the CH_4 – CO_2 – C_2H_4 system, covering all intermediates of the universal core of metabolism, and, as we noted earlier (Marakushev, 2008), light hydrocarbons (from methane to ethylene and its derivatives) could have

been a chemical source (and anaplerotic raw material) of the arising intermediates of CO_2 fixation cycles.

Thus, a “chemical space” of substances of the C–H–O system is a thermodynamically controlled network of reactions of intermediates, which creates modular structures that evolve under certain physicochemical conditions into specific chemoautotrophic systems of CO_2 fixation. However, the emerging autotrophic paleometabolism could have been somewhat different had the main source of carbon for the autocatalytic chemical networks been not carbon dioxide, but endogenous methane.

Autotrophic paleometabolism of methane fixation.

Recent studies of carbon isotope fractionation in ancient rocks have shown that the age of the first possible traces of life has significantly shifted towards

Table 1. Gibbs free energies for carboxylation, hydration, hydrogenation, and cleavage reactions in the TCA and 3-HP cycles and WL pathway in aqueous solutions at temperatures of 298 and 473 K and water vapor saturation pressure (P_{SAT})

	Reactions of intermediates of the CO ₂ fixation network	ΔG_{298}^0 , kJ	ΔG_{473}^0 , kJ
WL pathway	CO ₂ + H ₂ = CO + H ₂ O	11.04	13.51
	CO ₂ + CO + 3H ₂ = CH ₃ COOH(acetate) + H ₂ O	−180.54	−128.87
WL pathway, TCA cycle	CH ₃ COOH(acetate) + CO ₂ + H ₂ = CH ₃ (CO)COOH(pyruvate) + H ₂ O	45.46	71.91
	2CO₂ + 4H₂ = CH₃COOH(acetate) + 2H₂O	−169.50	−115.3
TCA cycle	CH ₃ (CO)COOH(pyruvate) + CO ₂ = CH ₂ CO(COOH) ₂ (oxaloacetate)	13.11	35.03
	CH ₂ CO(COOH) ₂ (oxaloacetate) + H ₂ = CH ₂ CH(OH)(COOH) ₂ (malate)	−65.49	−55.78
	CH ₂ CH(OH)(COOH) ₂ (malate) = (CH) ₂ (COOH) ₂ (fumarate) + H ₂ O	5.68	−5.26
	(CH) ₂ (COOH) ₂ (fumarate) + H ₂ = (CH ₂) ₂ (COOH) ₂ (succinate)	−102.24	−88.88
	(CH ₂) ₂ (COOH) ₂ (succinate) + CO ₂ + H ₂ = (CH ₂) ₂ CO(COOH) ₂ (2-oxoglutarate) + H ₂ O	17.90	47.89
	(CH ₂) ₂ CO(COOH) ₂ (2-oxoglutarate) + CO ₂ + H ₂ = (CH ₂) ₂ C(OH)(COOH) ₃ (citrate)	−18.11	1.82
	(CH ₂) ₂ CO(COOH) ₂ (2-oxoglutarate) + CO ₂ + H ₂ = (CH ₂) ₂ C(OH)(COOH) ₃ (isocitrate)	−15.69	—
	(CH ₂) ₂ C(OH)(COOH) ₃ (isocitrate) = C ₆ H ₆ O ₆ (cis-aconitate) + H ₂ O	2.36	—
	C ₆ H ₆ O ₆ (cis-aconitate) + H ₂ O = (CH ₂) ₂ C(OH)(COOH) ₃ (citrate)	−4.78	—
	(CH ₂) ₂ C(OH)(COOH) ₃ (citrate) = CH ₂ CO(COOH) ₂ (oxaloacetate) + CH ₃ COOH(acetate)	−7.24	−15.15
3-HP bicycle	CH ₃ COOH(acetate) + CO ₂ = CH ₂ (COOH) ₂ (malonate)	48.10	68.28
	CH ₂ (COOH) ₂ (malonate) + H ₂ = HCOCH ₂ COOH(malonate semialdehyde) + H ₂ O	−50.32	−49.96
	HCOCH ₂ COOH(malonate semialdehyde) + H ₂ = (OH)CH ₂ CH ₂ COOH(3-hydroxypropionate)	−43.93	−52.84
	(OH)CH ₂ CH ₂ COOH(3-hydroxypropionate) + H ₂ = CH ₃ CH ₂ COOH(propionate) + H ₂ O	−89.92	−61.88
	CH ₃ CH ₂ COOH(propionate) + CO ₂ = CHCH ₃ (COOH) ₂ (methylmalonate)	47.7	69.07
	CHCH ₃ (COOH) ₂ (methylmalonate) = (CH ₂) ₂ (COOH) ₂ (succinate)	−15.11	−15.66
	CH ₃ CH ₂ COOH(propionate) + HCOCOOH(glyoxylate) = CHCH ₃ CH(OH)(COOH) ₂ (methylmalate)	−74.74	−52.35
	CHCH ₃ CH(OH)(COOH) ₂ (methylmalate) = CCH ₃ CH(COOH) ₂ (mesaconate) + H ₂ O	5.64	−5.61
	CCH ₃ CH(COOH) ₂ (mesaconate) + H ₂ O = (OH)CCH ₃ CH ₂ (COOH) ₂ (citramalate)	30.05	39.29
	(OH)CCH ₃ CH ₂ (COOH) ₂ (citramalate) = CH ₃ (CO)COOH(pyruvate) + CH ₃ COOH(acetate)	19.35	1.73
	2CO₂ + 2H₂ = OCHCOOH(glyoxylate) + H₂O	31.73	69.88
PP cycle	CH ₃ (CO)COOH(pyruvate) + H ₃ PO ₄ = C ₃ H ₅ O ₆ P(phosphoenolpyruvate) + H ₂ O	875.73	917.27
	CH ₂ CO(COOH) ₂ (oxaloacetate) + H ₃ PO ₄ = C ₃ H ₅ O ₆ P + H ₂ O + CO ₂	865.62	882.24

The total reactions of the cycles are shown in bold. The phosphorylation reactions of pyruvate and oxaloacetate initiate the reductive pentose–phosphate (PP) pathway of CO₂ fixation. Thermodynamic constants from (Amend and Shock, 2001; Marakushev and Belonogova, 2012; Marakushev and Belonogova, 2013 (Suppl. Mat)) were used.

Hadean: 3.77 Ga (Dodd et al., 2017), 3.83 Ga (McKeegan et al., 2007), 3.95 Ga (Tashiro et al., 2017), 4.10 Ga (Bell et al., 2015) and, obviously, the environmental conditions at this time determined the evolutionary paths of the emerging metabolic networks. The continental crust and upper mantle of the early Earth were significantly more reduced than their modern counterparts (Yang et al., 2014), and methane was apparently the predominant gas in the hydro-

sphere and atmosphere (Pavlov et al., 2000; Tian et al., 2005; Zahnle et al., 2019). Gas–liquid inclusions of hydrocarbons and organic matter in Archean quartz (Touret, 2003; Schreiber et al., 2017) also indicate a enough reducing environment during this time period. It is possible that the high partial pressure of methane that existed in the hydrothermal systems of the ancient Earth was leading to the formation of primary methane assimilating autotrophic protometabolic systems.

Fig. 2. Phase space of intermediates of autotrophic metabolism on the diagram of the C–H–O composition system. Possible intermediates of chemoautotrophic CO₂ fixation systems are presented according to Morowitz and Meringer ((1) filled circles, outlined with a green dash–dotted line) (Morowitz et al., 2000; Meringer and Cleaves, 2017) and Zubarev ((2) squares, outlined with a black dotted line) (Zubarev et al., 2015). (3) Universal intermediates of paleometabolism (triangles, outlined in red) (Marakushev and Belonogova, 2009, 2010; Braakman and Smith 2012, 2013), (4) inclusions of hydrocarbons and simple organic substances in Archean quartz (stars, outlined in blue) (Schreiber et al., 2017).

A model of the primary ancient anaerobic methanotrophic pathway of acetogenesis, in which the carbon source is methane instead of CO₂, was proposed in (Nitschke and Russell, 2013; Russell and Nitschke, 2017), in which the reverse WL pathway (indicated in Fig. 1 by the number 1b) was proposed as a biomimetic basis for autotrophic metabolism. Activated nitric oxide (NO) formed during the nitrate/nitrite transformation is assumed to be the oxidizing agent of methane, and the authors suggest that this pathway of CH₄ fixation (“denitrifying methanotrophic acetogenesis”) was the first energy system of metabolism in the hydrothermal emissions of the early Earth.

Recent studies have shown that the Archean *Methanosarcina acetivorans* forms acetate in the reverse WL path when methane oxidation is coupled with the reduction of iron(III) (Soo et al., 2016; Timmers et al., 2017; Yan et al., 2018). The stoichiometry of the reverse WL pathway reaction in archaea suggests a path in which four methane molecules are oxidized and two CO₂ molecules are reduced to form three acetate molecules (Soo et al., 2016). This pathway of carboxy-methanotrophic acetogenesis can also be considered as a biomimetic model of the primary metabolic system of CH₄ fixation. This pathway is thermodynamically very advantageous using NO as an oxidizing agent: CH₄ + 0.5CO₂ + 2NO + H₂ =

0.75CH₃COOH + N₂ + 1.5H₂O, $\Delta G_{298}^0 = -629.17$; $\Delta G_{473}^0 = -592.04$ kJ/mol CH₄ and is quite favorable in coupling with the reduction of ferric iron—a component of the mineral hematite: CH₄ + 0.5CO₂ + 6Fe₂O₃ (hematite) + H₂ = 0.75CH₃COOH + 4Fe₃O₄ (magnetite) + 1.5H₂O, $\Delta G_{298}^0 = -30.54$; $\Delta G_{473}^0 = -35.34$ kJ/mol CH₄.

The initiating step in the anaerobic oxidation of both aromatic and aliphatic hydrocarbons is their binding with fumarate (Haynes and Gonzalez, 2014), and the reaction of methane with fumarate C₄H₄O₄ (fumarate) + CH₄ = C₅H₈O₄ (2-methylsuccinate) satisfies the energy requirements for autotrophic growth (Thauer and Shima 2008; Beasley and Nunny, 2012; Aversch and Kracke, 2018). This suggests the possibility of its participation in the incipient autotrophic metabolism (Marakushev and Belonogova, 2019), precisely within the above-discussed universal “chemical space” of intermediates: carboxy- and α-keto acids.

Let us consider the construction of a metabolic network that combines a part of the above universal core of paleometabolism (sequence of the TCA cycle) (Fig. 1), with the supposed methano-fumarate (MF) cycle (Marakushev and Belonogova, 2019), as a model of methanotrophic metabolism (Fig. 3), which origi-

Fig. 3. Scheme of coupling of the methane–fumarate (MF) cycle (I, bold arrows) with the TCA cycle of CO₂ fixation (II) based on the general sequence of reactions oxaloacetate → malate → fumarate. Methane carbon is incorporated into fumarate, and CO₂ carbon is incorporated into pyruvate, succinate, and 2-oxoglutarate with the formation of a C–C bond. Fumarate is a bifurcation point towards hydrogenotrophic (succinate formation) or methanotrophic (2-methyl succinate formation) metabolism.

nated and functioned in the reducing hydrothermal systems of the early Archean at a high partial pressure of methane. A combination of a part of the TCA cycle of CO₂ fixation with the methane–fumarate branch of CH₄ fixation is presented as the supposed chemical basis of primary autotrophic paleometabolism (TCA–MF bicycle). In this chemical symbiosis of cycles, the bifurcation point is fumarate, which is transformed into succinate (initiation of the TCA cycle) or 2-methyl succinate (initiation of the MF cycle).

Assimilation of methane is carried out by binding methane with fumarate to form 2-methyl succinate, the anaerobic oxidation of which leads to the formation of acetate and pyruvate. The formation of pyruvate (the central “hub” of intermediate metabolism) opens the way for the introduction of methane carbon into the universal chemical space of intermediates of autotrophic metabolism. Pyruvate assimilates CO₂ with the formation of oxaloacetate, which is transformed into fumarate in the reactions of the compo-

nents of the reductive citrate cycle. Fumarate, again assimilating methane, starts a new autocatalytic MF cycle, in one turn of which an acetate molecule is formed from methane and carbon dioxide molecules. The sequence of reactions of dicarboxylic acids, oxaloacetate → malate → fumarate → succinate, common for the TCA and MF cycles, was recently demonstrated experimentally with protonated intermediates under catalysis by a combination of native iron with Zn²⁺ and Cr³⁺ ions (Varma et al., 2018). The problem of the most energetically unfavorable reaction of transformation of 2-methyl succinate into citramalate ($\Delta G_{298}^0 = 96.57$ kJ/mol, Table 2) can be solved by using in the reaction oxidizing agents such as nitrogen and iron oxides. Anaerobic fixation of methane in the MF cycle can be represented in the form of the reactions $C_4H_4O_4$ (fumarate) + CH₄ + [O] = C₂H₄O₂ (acetate) + C₃H₄O₃ (pyruvate), where [O] is an inorganic oxidant. The free energy of reactions with the

Table 2. Free energies of the reactions of the MF branch (I) of the bicycle and the total reaction of CH₄ fixation with the participation of oxidized forms of nitrogen and iron as oxidants under hydrothermal conditions at temperatures of 298 and 473 K and P_{SAT}

MF cycle reactions	ΔG_{298}^0 , kJ	ΔG_{473}^0 , kJ
$(\text{CH})_2(\text{COOH})_2(\text{fumarate}) + \text{CH}_4 = (\text{CH}_2)(\text{CH}_3\text{CH})(\text{COOH})_2(\text{methyl succinate})$	−44.95	−29.97
$(\text{CH}_2)(\text{CH}_3\text{CH})(\text{COOH})_2(\text{methyl succinate}) + \text{H}_2\text{O} = (\text{CH}_3\text{CH})\text{CH}(\text{OH})(\text{COOH})_2(\text{citramalate}) + \text{H}_2$	96.57	94.14
$(\text{CH}_3\text{C}=\text{CH})(\text{COOH})_2(\text{mesaconate}) + \text{H}_2 = (\text{CH}_2)(\text{CH}_3\text{CH})(\text{COOH})_2(\text{methyl succinate})$	−66.53	−54.85
$(\text{CH}_3\text{C}=\text{CH})(\text{COOH})_2(\text{mesaconate}) + \text{H}_2\text{O} = (\text{CH}_3\text{CH})\text{CH}(\text{OH})(\text{COOH})_2(\text{citramalate})$	30.05	39.29
$(\text{CH}_3\text{CH})\text{CH}(\text{OH})(\text{COOH})_2(\text{citramalate}) = \text{C}_2\text{H}_4\text{O}_2(\text{acetate}) + \text{C}_3\text{H}_4\text{O}_3(\text{pyruvate})$	19.35	1.73
Total fixation of methane		
$\text{C}_4\text{H}_4\text{O}_4(\text{fumarate}) + \text{CH}_4 + \text{H}_2\text{O} = \text{C}_2\text{H}_4\text{O}_2(\text{acetate}) + \text{C}_3\text{H}_4\text{O}_3(\text{pyruvate}) + \text{H}_2$	70.97	65.9
$\text{C}_4\text{H}_4\text{O}_4 + \text{CH}_4 + 0.5\text{O}_2 = \text{C}_2\text{H}_4\text{O}_2 + \text{C}_3\text{H}_4\text{O}_3$	−192.22	−182.53
$\text{C}_4\text{H}_4\text{O}_4 + \text{CH}_4 + 2\text{HNO}_2 = \text{C}_2\text{H}_4\text{O}_2 + \text{C}_3\text{H}_4\text{O}_3 + \text{H}_2\text{O} + 2\text{NO}$	−115.77	−143.28
$\text{C}_4\text{H}_4\text{O}_4 + \text{CH}_4 + 2\text{NO} = \text{C}_2\text{H}_4\text{O}_2 + \text{C}_3\text{H}_4\text{O}_3 + \text{N}_2\text{O}$	−274.7	−250.83
$\text{C}_4\text{H}_4\text{O}_4 + \text{CH}_4 + \text{NO} = \text{C}_2\text{H}_4\text{O}_2 + \text{C}_3\text{H}_4\text{O}_3 + 0.5\text{N}_2$	−276.93	−264.48
$\text{C}_4\text{H}_4\text{O}_4 + \text{CH}_4 + \text{Fe}_3\text{O}_4 + 1.5\text{SiO}_2 = \text{C}_2\text{H}_4\text{O}_2 + \text{C}_3\text{H}_4\text{O}_3 + 1.5\text{Fe}_2\text{SiO}_4$	45.08	25.21
$\text{C}_4\text{H}_4\text{O}_4 + \text{CH}_4 + 3\text{Fe}_2\text{O}_3 = \text{C}_2\text{H}_4\text{O}_2 + \text{C}_3\text{H}_4\text{O}_3 + 2\text{Fe}_3\text{O}_4$	22.38	13.87
$\text{C}_4\text{H}_4\text{O}_4 + \text{CH}_4 + 0.75\text{FeS}_2 + 0.25\text{Fe}_3\text{O}_4 = \text{C}_2\text{H}_4\text{O}_2 + \text{C}_3\text{H}_4\text{O}_3 + 1.5\text{FeS}$	38.78	31.54

Free energies of aqueous substances formation according to (Amend and Shock, 2001; Marakushev and Belonogova, 2013 (Suppl. Mat.)).

participation of oxidized forms of nitrogen and iron is given in Table 2. The autocatalytic nature of the MF cycle is associated with the branching of citramalate into pyruvate and acetate and can be expressed as the reaction $\text{C}_4\text{H}_6\text{O}_5$ (malate) + 1.5CH_4 + 2.5CO_2 = $2\text{C}_4\text{H}_6\text{O}_5$ (two malates). This type of autotrophic metabolism, as in the case of the aforementioned reverse WL pathway, can be defined as *carboxy-methanotrophic acetogenesis* (Table 2).

The origin and evolution of chemical systems of paleometabolism was determined by the physico-chemical conditions of existence of the ancient hydrothermal system, the model of which is presented in the form of a phase diagram of the chemical potential of oxygen–temperature (Fig. 4). The diagram is a two-component system (C and H are extensive parameters), since oxygen, represented by the logarithm of the O₂ activity in solution, passes into the number of intensive parameters along with temperature and pressure. Accordingly, at an arbitrary pressure, nonvariant equilibria on the diagram (points) consist of four phases, and three-phase equilibria (lines) separate the divariant stability fields (facies) of two-phase equilibria.

The diagram is divided by the equilibrium $\text{CH}_4 + 2\text{O}_2 = \text{CO}_2 + 2\text{H}_2\text{O}$ (bold line) into two phase spaces, designated by Roman numerals I and II, corresponding to the oxidizing and reducing conditions of the hydrothermal system. Metastable equilibria form the phase spaces of stability (facies) of parageneses (asso-

ciations) of the intermediates of the TCA-MF bicycle—acetate, succinate, and fumarate. The fumarate and succinate facies are located on both sides of the stable $\text{CH}_4 \leftrightarrow \text{CO}_2$ equilibrium; however, the succinate facies is limited to the temperature of 549 K. In a hydrothermal solution, the parageneses of the components of the fumarate cycle are stable both in the CO₂ facies and in the CH₄ facies; i.e., they can develop systems for carbon fixation assimilable in the form of CO₂ or CH₄. The acetate facies completely covers the equilibrium $\text{CH}_4 + \text{O}_2 = \text{CO}_2 + \text{H}_2\text{O}$, and the entire system, with a change in the chemical potential of oxygen, can develop towards the formation of low-temperature (Suc–H₂O) and high-temperature (Fum–H₂O) paragenesis in the CO₂ facies (I) or the formation of low-temperature (Suc–CH₄) and high temperature (Fum–CH₄) paragenesis in the CH₄ (II) facies. Thus, the methane facies (II) is a wide range of thermodynamic stability of systems for the assimilation of CH₄ by organic acids and keto acids in an aqueous environment.

Mineral buffers that determine the redox environment up to a temperature of 549 K are located in the succinate facies; however, the equilibrium hematite–magnetite (HM) is in the region of thermodynamic stability of CO₂ (facies I) but pyrite–pyrrhotite–magnetite (PPM) and quartz–magnetite–fayalite (QMF) equilibria in facies II (methane stability). The last two

Fig. 4. The range of changes in the redox state (O_2 activity) of the continental crust of the Archean 3.8 Ga (shaded green area) on the diagram logarithm of oxygen activity ($\log a_{O_2}$) and temperature (T , K) under hydrothermal conditions at P_{SAT} . The phase spaces of the thermodynamic stability of substances and their assemblages were calculated according to the method of (Marakushev and Belonogova, 2009). The bold line is the $CO_2 \leftrightarrow CH_4$ equilibrium separating the regions of their thermodynamic stability (I and II). The succinate facies is outlined by blue equilibrium lines, and the acetate facies is outlined in red. Dashed lines are equilibria of mineral buffers: hematite–magnetite, Fe_2O_3/Fe_3O_4 (HM), pyrite–pyrrhotite–magnetite, $FeS_2 + Fe_3O_4/FeS$ (PPM) and quartz–magnetite–fayalite, $SiO_2 + Fe_3O_4/Fe_2SiO_4$ (QMF). ΔQMF is the range of change relative to the equilibrium of the QMF buffer in logarithmic units of O_2 activity. Designations: Acet, acetate; Suc, succinate; Fum, fumarate. Free energy designation of aqueous substances according to (Amed and Shock, 2001).

equilibria determine the temperature redox conditions for the fundamental equilibrium $2Suc + 2CH_4 + O_2 = 5Acet$, as the basis of methanotrophic acetogenesis. The magnetite facies (Fe_3O_4) covers the $CH_4 \leftrightarrow CO_2$ equilibrium practically over the entire temperature range of the hydrothermal system under consideration. Obviously, the CO_2 fixation systems should have developed above the $CO_2 \leftrightarrow CH_4$ equilibrium, while the CH_4 fixation systems should have developed below it. Apparently, the simultaneous fixation of CO_2 and CH_4 with the formation of acetate (carboxy-methanotrophy) (Fig. 3) occurred in the magnetite facies (Fe_3O_4), and both of these substrates could be a source of carbon for the development of paleometabolism in the area of thermodynamic stability of dicarboxylic acids—universal substances of intermediate metabolism.

Based on the analysis of trace elements of magmatic zircons of crustal origin (mainly data from a cerium-based oxybarometer), it was shown that the continental crust of Hadean was significantly more reduced than the modern one and underwent progressive oxidation in the early Archean ~3.6 Ga ago (Yang et al., 2014). During this period of the possible origin

of life, the redox state of the Earth's crust ($\log f_{O_2}$) periodically changed relative to the equilibrium of the fayalite–magnetite–quartz buffer (ΔQMF). For example, in zircons with an age of 3.8 Ga, ΔQMF ranged from -6.0 to $+5.5$, and this redox range is shown in the diagram (Fig. 4). This space completely encompasses both the CH_4/CO_2 equilibrium and the magnetite facies, but to a greater extent belongs to the low-temperature reducing methane facies (II), which includes all equilibria of methane assimilation under consideration. Up to 3.6 Ga and, perhaps, even before the great oxidation event (GOE) 2.2–2.4 Ga, the oxidation potential of magnetite facies in the Earth's crust apparently determined the chemical potential of oxygen in ancient submarine mineralogical systems. Thus, the considered hydrothermal redox and P - T conditions of the Early Archean are extremely favorable for the development of methanotrophic and carboxy–methanotrophic systems of paleometabolism.

CONCLUSIONS

The set of universal intermediates of autotrophic paleometabolism forms the phase space of substances of the C–H–O system, the chemical base of which is

light hydrocarbons. A certain “chemical space” of substances is a thermodynamically controlled network of intermediates, the combination of which created various systems of autotrophic paleometabolism. Modern autotrophic pathways for carbon fixation were apparently formed as a result of a combination of individual modules of metabolic systems created by ancestral metabolism, the reversibility of reactions of which allowed us to implement various strategies for realization autotrophic carbon assimilation.

Autotrophic metabolism presupposes the assimilation of inorganic carbon exclusively in the form of CO₂; however, methane is also a abyssal, inorganic substance, and, therefore, its fixation is also a manifestation of autotrophic metabolism. Anaerobic fixation of methane by universal components of protometabolic networks under hydrothermal conditions is thermodynamically favorable. Under the conditions of hydrocarbon degassing of the early Earth, carbon fixation in the form of hydrocarbons could have predominated, but with the transition of our planet to the CO₂ degassing regime, relic forms of methanotrophy inevitably had to either die out or evolve into methanogenic forms or be thrown into extreme ecological niches. Even ancient microfossils may not be directly related to LUCA and its descendants, but are the remains of other extinct ancestors. If we assume the existence of earlier ancestors before LUCA (Cornish-Bowden and Cárdenas, 2017), then the number of carbon-fixation metabolic systems in the putative populations of pre-LUCA organisms should be much larger than is currently known. It is also possible that the modern pathways of methanotrophy are relics of the paleometabolism of the Archean methanotrophic superiority of prokaryotes. The study of the “chemical space” of protometabolic networks and extrapolation of the results to the conditions of nascent life is part of molecular paleontology, and many still unclear answers to fundamental questions about the origin of ancient metabolism may be hidden in the composition and architecture of modern biochemical reaction networks.

FUNDING

This work was conducted on the topic of a State Assignment, project no. AAAA-A19-119071190045-0.

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REFERENCES

- Amend, J.P. and Shock, E.L., Energetics of overall metabolic reactions of thermophilic and hyperthermophilic archaea and bacteria, *FEMS Microbiol. Rev.*, 2001, vol. 25, pp. 175–243.
- Averesch, N.J.H. and Kracke, F., Metabolic network analysis of microbial methane utilization for biomass formation and upgrading to bio-fuels, *Front. Energy Res.*, 2018, vol. 6, pp. 1–16.
- Beasley, K.K. and Nanny, M.A., Potential energy surface for anaerobic oxidation of methane via fumarate addition, *Environ. Sci. Technol.*, 2012, vol. 46, pp. 8244–8252.
- Bell, E.A., Boehnke, P., Harrison, T.M., and Mao, W.L., Potentially biogenic carbon preserved in a 4.1 billion-year-old zircon, *Proc. Natl. Acad. Sci. U. S. A.*, 2015, vol. 112, pp. 14518–14521.
- Braakman, R. and Smith, E., The emergence and early evolution of biological carbon-fixation, *PLoS Comput. Biol.*, 2012, vol. 8, pp. 1–16.
- Braakman, R. and Smith, E., The compositional and evolutionary logic of metabolism, *Phys. Biol.*, 2013, vol. 10, pp. 1–63.
- Catling, D.C. and Kasting, J.F., *Atmospheric Evolution on Inhabited and Lifeless Worlds*, Cambridge: Cambridge Univ. Press, 2017.
- Cornish-Bowden, A. and Cárdenas, M.L., Life before LUCA, *J. Theor. Biol.*, 2017, vol. 434, pp. 68–74.
- Dodd, M.S., Papineau, D., Grenne, T., Slack, J.F., Rittner, M., Pirajno, F., O’Neil, J., and Little, C.T.S., Evidence for early life in earth’s oldest hydrothermal vent precipitates, *Nature*, 2017, vol. 543, pp. 60–64.
- Goldford, J.E., Hartman, H., Smith, T.F., and Segre, D., Remnants of an ancient metabolism without phosphate, *Cell*, 2017, vol. 168, pp. 1–9.
- Haynes, C.A. and Gonzalez, R., Rethinking biological activation of methane and conversion to liquid fuels, *Nat. Chem. Biol.*, 2014, vol. 10, pp. 331–339.
- Hordijk, W. and Steel, M., Autocatalytic networks at the basis of life’s origin and organization, *Life*, 2018, vol. 8, pp. 62–73.
- Hügler, M. and Sievert, S.M., Beyond the Calvin cycle: autotrophic carbon fixation in the ocean, *Ann. Rev. Mar. Sci.*, 2011, vol. 3, pp. 261–289.
- Mall, A., Sobotta, J., Huber, C., Tschirner, C., Kowarschik, S., Bačnik, K., Mergelsberg, M., Boll, M., Hügler, M., Eisenreich, W., and Berg, I., A reversibility of citrate synthase allows autotrophic growth of a thermophilic bacterium, *Science*, 2018, vol. 359, pp. 563–567.
- Marakushev, S.A., Transformation of hydrocarbons into components of archaic chemoautotrophic CO₂ fixation cycle, *Dokl. Biochem. Biophys.*, 2008, vol. 418, no. 3, pp. 18–23.

- Marakushev, S.A. and Belonogova, O.V., The parageneses thermodynamic analysis of chemoautotrophic CO₂ fixation archaic cycle components, their stability and self-organization in hydrothermal systems, *J. Theor. Biol.*, 2009a, vol. 257, pp. 588–597.
- Marakushev, S.A. and Belonogova, O.V., Physicochemical conditions for the formation and self-organization of components of the archaic cycle of CO₂ fixation in hydrothermal systems, *Biofizika*, 2009b, vol. 54, no. 4, pp. 748–759.
- Marakushev, S.A. and Belonogova, O.V., Evolution of carbon dioxide archaic chemoautotrophic fixation system in hydrothermal systems, *Dokl. Biochem. Biophys.*, 2010, vol. 433, no. 2, pp. 168–174.
- Marakushev, S.A. and Belonogova, O.V., Thermodynamic factors of natural selection in autocatalytic chemical systems, *Dokl. Biochem. Biophys.*, 2012, vol. 444, no. 1, pp. 131–136.
- Marakushev, S.A. and Belonogova, O.V., The divergence and natural selection of autocatalytic primordial metabolic systems, *Orig. Life Evol. Biosph.*, 2013, vol. 43, pp. 263–281.
- Marakushev, S.A. and Belonogova, O.V., The origin of ancestral bacterial metabolism, *Paleontol. J.*, 2013a, vol. 47, no. 9, pp. 1001–1009.
- Marakushev, S.A. and Belonogova, O.V., The origin of primitive chemoautotrophic metabolism in hydrotherms and the emergence of ancestral taxa of prokaryotes, in *Problemy zarozhdeniya i evolyutsii biosfery* (Problems of the Origin and Evolution of the Biosphere), Galimov, E.M., Ed., Moscow: URSS, 2013b, pp. 414–433.
- Marakushev, S.A. and Belonogova, O.V., Ideas and perspectives: development of nascent autotrophic carbon fixation systems in various redox conditions of the fluid degassing on early Earth, *Biogeosciences*, 2019, vol. 16, pp. 1817–1828.
- Martin, W. and Russell, M.J., On the origin of biochemistry at an alkaline hydrothermal vent, *Philos. Trans. R. Soc., B*, 2007, vol. 362, pp. 1887–1926.
- Martin, W.F., Weiss, M.C., Neukirchen, S., Nelson-Sathi, S., and Sousa, F., L physiology, phylogeny, and LUCA, *Microb. Cell*, 2016, vol. 3, pp. 582–587.
- McKeegan, K.D., Kudryavtsev, A.B., and Schopf, J.W., Raman and ion microscopic imagery of graphitic inclusions in apatite from the >3830 Ma Akilia supracrustals, West Greenland, *Geology*, 2007, vol. 35, pp. 383–397.
- Meringer, M. and Cleaves, J., Computational exploration of the chemical structure space of possible reverse tricarboxylic acid cycle constituents, *Sci. Rep.*, 2017, vol. 7, pp. 1–12.
- Morowitz, H.J., Kostelnik, J.D., Yang, J., and Cody, G.D., The origin of intermediary metabolism, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, vol. 97, pp. 7704–7708.
- Muchowska, K.B., Varma, S.J., Chevallot-Beroux, E., Lethuillier-Karl, L., Li, G., and Moran, J., Metals promote sequences of the reverse Krebs cycle, *Nat. Ecol. Evol.*, 2017, vol. 1, pp. 1716–1721.
- Muchowska, K.B., Chevallot-Beroux, E., and Moran, J., Recreating ancient metabolic pathways before enzymes, *Bioorg. Med. Chem.*, 2019, vol. 27, pp. 2292–2297.
- Nitschke, W. and Russell, M.J., Beating the acetyl-CoA pathway to the origin of life, *Philos. Trans. R. Soc., B*, 2013, vol. 368, art. 20120258.
- Nunoura, T., Chikaraishi, Y., Izaki, R., Suwa, T., Sato, T., Harada, T., Mori, K., Kato, Y., Miyazaki, M., Shimamura, S., Yanagawa, K., Shuto, A., Ohkouchi, N., Fujita, N., Takaki, Y., Atomi, H., and Takai, K., A primordial and reversible TCA cycle in a facultatively chemolithoautotrophic thermophile, *Science*, 2018, vol. 359, pp. 559–563.
- Nutman, A.P., Bennett, V.C., Friend, C.R.L., Van Kranendonk, M.J., and Chivas, A.R., Rapid emergence of life shown by discovery of 3,700-million-year-old microbial structures, *Nature*, 2016, vol. 537, pp. 535–539.
- Pavlov, A.A., Kasting, J.F., Brown, L.L., Rages, K.A., and Freedman, R., Greenhouse warming by CH₄ in the atmosphere of early Earth, *J. Geophys. Res.*, 2000, vol. 105, pp. 11981–11990.
- Potter, J. and Konnerup-Madsen, J., A review of the occurrence and origin of abiogenic hydrocarbons in igneous rocks, in *Hydrocarbons in Crystalline Rocks*, Petford, N. and McCaffrey, K.J.W., Eds., Geol. Soc. London, Special Publications, 2003, vol. 214, pp. 151–173.
- Ralsler, M., An appeal to magic? The discovery of a non-enzymatic metabolism and its role in the origins of life, *Biochem. J.*, 2018, vol. 475, pp. 2577–2592.
- Ruiz-Mirazo, K., Briones, C., and de la Escosura, A., Chemical roots of biological evolution: the origins of life as a process of development of autonomous functional systems, *Open Biol.*, 2017, vol. 7, p. 170050.
- Russell, M.J. and Martin, W., The rocky roots of the acetyl-CoA pathway, *Trends Biochem. Sci.*, 2004, vol. 29, pp. 358–363.
- Russell, M.J. and Nitschke, W., Methane: fuel or exhaust at the emergence of life?, *Astrobiology*, 2017, vol. 17, pp. 1053–1066.
- Schreiber, U., Mayer, C., Schmitz, O.J., Rosendahl, P., Bronja, A., Greule, M., Keppler, F., Mulder, I., Sattler, T., and Schöler, H.F., Organic compounds in fluid inclusions of Archean quartz—analogs of prebiotic chemistry on early earth, *PLoS One*, 2017, vol. 12, e0177570.
- Smith, E. and Morowitz, H.G., Universality in intermediary metabolism, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, vol. 101, pp. 13168–13173.
- Soo, V.W.C., McAnulty, M.J., Tripathi, A., Zhu, F., Zhang, L., Hatzakis, E., Smith, P., Agrawal, S., Nazem-Bokaei, H., Gopalakrishnan, S., Salis, H.M., Ferry, J.G., Maranas, C.D., Patterson, A.D., and Wood, T.K., Reversing methanogenesis to capture methane for liquid biofuel precursors, *Microb. Cell Fact.*, 2016, vol. 15, pp. 11–25.
- Tashiro, T., Ishida, A., and Hori, M., Early trace of life from 3.95 Ga sedimentary rocks in Labrador, Canada, *Nature*, 2017, vol. 549, pp. 516–518.
- Thauer, R.K. and Shima, S., Methane as fuel for anaerobic microorganisms, *Ann. N.Y. Acad. Sci.*, 2008, vol. 1125, pp. 158–170.
- Tian, F., Toon, O.B., Pavlov, A.A., and De Sterck, H., A hydrogen-rich early Earth atmosphere, *Science*, 2005, vol. 308, pp. 1014–1017.

- Timmers, P.H.A., Welte, C.U., Koehorst, J.J., Plugge, C.M., Jetten, M.S.M., and Stams, A.J.M., Reverse methanogenesis and respiration in methanotrophic Archaea, *Archaea*, 2017, vol. 17, pp. 1–22.
- Touret, J.L.R., Remnants of early Archaean hydrothermal methane and brines in pillow-breccia from the Isua-Greenstone Belt, West Greenland, *Precambrian Res.*, 2003, vol. 126, pp. 219–233.
- Varma, S.J., Muchowska, K., Chatelain, P., and Moran, J., Native iron reduces CO₂ to intermediates and end-products of the acetyl-CoA pathway, *Nat. Ecol. Evol.*, 2018, vol. 2, pp. 1019–1024.
- Wächtershäuser, G., Evolution of the first metabolic cycles, *Proc. Natl. Acad. Sci. U. S. A.*, 1990, vol. 87, pp. 200–204.
- Wächtershäuser, G., Groundworks for an evolutionary biochemistry—the iron-sulfur world, *Prog. Biophys. Mol. Biol.*, 1992, vol. 58, pp. 85–201.
- Weiss, M.C., Sousa, F.L., Mrnjavac, N., Neukirchen, S., Roettger, M., Nelson-Sathi, S., and Martin, W.F., The physiology and habitat of the last universal common ancestor, *Nat. Microbiol.*, 2016, vol. 1, p. 16116.
- Weiss, M.C., Preiner, M., Xavier, J.C., Zimorski, V., and Martin, W.F., The last universal common ancestor between ancient Earth chemistry and the onset of genetics, *PLoS Genet.*, 2018, vol. 14, no. 8. e1007518.
- Yan, Z., Joshi, P., Gorski, C.A., and Ferry, J.G., A biochemical framework for anaerobic oxidation of methane driven by Fe(III)-dependent respiration, *Nat. Commun.*, 2018, vol. 9, no. 1642, pp. 1–9.
- Yang, X., Gaillard, F., and Scaillet, B., A relatively reduced hadean continental crust and implications for the early atmosphere and crustal rheology, *Earth Planet. Sci. Lett.*, 2014, vol. 393, pp. 210–219.
- Zahnle, K.J., Gacesa, M., and Catling, D.C., Strange messenger: a new history of hydrogen on earth, as told by xenon, *Geochim. Cosmochim. Acta*, 2019, vol. 244, pp. 56–85.
- Zubarev, D.Y., Rappoport, D., and Aspuru-Guzik, A., Uncertainty of prebiotic scenarios: the case of the non-enzymatic reverse tricarboxylic acid cycle, *Sci. Rep.*, 2015, vol. 5, no. 8009, pp. 1–7.

Translated by P. Kuchina