Chemical Basis of Carbon Fixation Autotrophic Paleometabolism

S. A. Marakushev*, and O. V. Belonogova*, **

*a Institute of Problems of Chemical Physics, Russian Academy of Sciences, Chernogolovka, Moscow region, 142432 Russia

*e-mail: marak@cat.icp.ac.ru

**e-mail: ovbel@icp.ac.ru

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 subsequently 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 CO$_2$ fixation, was proposed and substantiated as a model of the primary anabolic chemical system of CO$_2$ 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 CO$_2$ fixation (Russell and Martin, 2004; Martin and Russell, 2007; Weiss et al., 2016; etc.) and the 3-HP bicycle of CO$_2$ 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 CO$_2$ 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 phylogenetic 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 CO$_2$ 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: CH$_3$COOH (acetate) + CO$_2$ + H$_2$ = CH$_3$(CO)COOH (pyruvate) + H$_2$O or initiates a 3-HP bicycle: CH$_3$COOH (acetate) + CO$_2$=CH$_3$(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 _Nitrispirae_, 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 $(G^{298}_2 = ±102.24$ 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 CO$_2$ 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 CO$_2$ 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–H$_2$O 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-
nogova, 2009, 2010; Braakman and Smith, 2012, 2013; Goldford et al., 2017). On the С–Н–О composition diagram (Fig. 2), they are located inside the triangle with the СН4–С2Н4 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 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 СН4–CO2–С2Н4 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 CO2 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 CO2 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 ($P_{\text{SAT}}$).

| Reactions of intermediates of the CO₂ fixation network | $\Delta G^\circ_{298}$ kJ | $\Delta G^\circ_{473}$ kJ |
|-------------------------------------------------------|--------------------------|--------------------------|
| WL pathway                                            |                          |                          |
| $\text{CO}_2 + \text{H}_2 = \text{CO} + \text{H}_2\text{O}$ | 11.04                    | 13.51                    |
| $\text{CO}_2 + \text{CO} + 3\text{H}_2 = \text{CH}_3\text{COOH}(\text{acetate}) + \text{H}_2\text{O}$ | -180.54                   | -128.87                   |
| WL pathway, TCA cycle                                  |                          |                          |
| $\text{CH}_3\text{COOH}(\text{acetate}) + \text{CO}_2 + \text{H}_2 = \text{CH}_3(\text{CO})\text{COOH}(\text{pyruvate}) + \text{H}_2\text{O}$ | 45.46                    | 71.91                    |
| $2\text{CO}_2 + 4\text{H}_2 = \text{CH}_3\text{COOH}(\text{acetate}) + 2\text{H}_2\text{O}$ | -169.50                   | -115.3                    |
| TCA cycle                                             |                          |                          |
| $\text{CH}_3(\text{CO})\text{COOH}(\text{pyruvate}) + \text{CO}_2 = \text{CH}_3(\text{CO})\text{COOH}(\text{oxaloacetate})$ | 13.11                    | 35.03                    |
| $\text{CH}_2\text{CO}(\text{COOH})_2(\text{oxaloacetate}) + \text{H}_2 = \text{CH}_2(\text{CH}_2\text{OH})(\text{COOH})_2(\text{malate})$ | -65.49                   | -55.78                   |
| $\text{CH}_3\text{CH}(\text{OH})(\text{COOH})_2(\text{malate}) = (\text{CH}_2)_2(\text{COOH})_2(\text{fumarate}) + \text{H}_2\text{O}$ | 5.68                     | -5.26                    |
| $(\text{CH}_2)_2(\text{COOH})_2(\text{fumarate}) + \text{H}_2 = (\text{CH}_2)_2(\text{COOH})_2(\text{succinate})$ | -102.24                  | -88.88                   |
| $(\text{CH}_2)_2(\text{COOH})_2(\text{succinate}) + \text{CO}_2 + \text{H}_2 = (\text{CH}_2)_2(\text{CO}(\text{COOH})_2(2\text{-oxoglutarate}) + \text{H}_2\text{O}$ | 17.90                    | 47.89                    |
| $(\text{CH}_2)_2(\text{CO}(\text{COOH})_2(2\text{-oxoglutarate}) + \text{CO}_2 + \text{H}_2 = (\text{CH}_2)_2(\text{C}(\text{OH})(\text{COOH})_2(\text{citrate}$ | -18.11                   | 1.82                     |
| $(\text{CH}_2)_2(\text{CO}(\text{COOH})_2(2\text{-oxoglutarate}) + \text{CO}_2 + \text{H}_2 = (\text{CH}_2)_2(\text{C}(\text{OH})(\text{COOH})_2(\text{isocitrate}$ | -15.69                   | -                    |
| $(\text{CH}_2)_2(\text{C}(\text{OH})(\text{COOH})_2(\text{isocitrate}) = (\text{CH}_4\text{O}_6(\text{cis}-\text{aconitate}) + \text{H}_2\text{O}$ | 2.36                     | -                    |
| $\text{C}_4\text{H}_6\text{O}_6(\text{cis}-\text{aconitate}) + \text{H}_2\text{O} = (\text{CH}_2)_2(\text{C}(\text{OH})(\text{COOH})_2(\text{citrate}$ | -4.78                    | -                    |
| $(\text{CH}_2)_2(\text{C}(\text{OH})(\text{COOH})_2(\text{citrate}) = \text{CH}_3(\text{CO})(\text{COOH})_2(\text{oxaloacetate}) + \text{CH}_3\text{COOH}(\text{acetate}$ | -7.24                    | -15.15                   |
| 3-HP bicycle                                           |                          |                          |
| $\text{CH}_3\text{COOH}(\text{acetate}) + \text{CO}_2 = \text{CH}_2(\text{COOH})_2(\text{malonate})$ | 48.10                    | 68.28                    |
| $\text{CH}_2(\text{COOH})_2(\text{malonate}) + \text{H}_2 = \text{HCOCH}_2\text{COOH}(\text{malonate semialdehyde}) + \text{H}_2\text{O}$ | -50.32                   | -49.96                   |
| $\text{HCOCH}_2\text{COOH}(\text{malonate semialdehyde}) + \text{H}_2 = (\text{OH})\text{CH}_2\text{CH}_2\text{COOH}(3\text{-hydroxypropionate})$ | -43.93                   | -52.84                   |
| $(\text{OH})\text{CH}_2\text{CH}_2\text{COOH}(3\text{-hydroxypropionate}) + \text{H}_2 = \text{CH}_3\text{CH}_2\text{COOH}(\text{propionate}) + \text{H}_2\text{O}$ | -89.92                   | -61.88                   |
| $\text{CH}_3\text{CH}_2\text{COOH}(\text{propionate}) + \text{CO}_2 = \text{CHCH}_3(\text{COOH})_2(\text{methylmalonate})$ | 47.7                     | 69.07                    |
| $\text{CHCH}_3(\text{COOH})_2(\text{methylmalonate}) = (\text{CH}_2)_2(\text{COOH})_2(\text{succinate})$ | -15.11                   | -15.66                   |
| $\text{CH}_3\text{CH}_2\text{COOH}(\text{propionate}) + \text{HCOCOOH}(\text{glyoxylate}) = \text{CHCH}_3(\text{CH}(\text{OH})(\text{COOH})_2(\text{methylmalate})$ | -74.74                   | -52.35                   |
| $\text{CHCH}_3(\text{OH})(\text{COOH})_2(\text{methylmalate}) = \text{CHCH}_3(\text{CH}(\text{OH})(\text{COOH})_2(\text{methylmalonate})$ | 5.64                     | -5.61                    |
| $\text{CCH}_3(\text{CH}(\text{OH})(\text{COOH})_2(\text{methylmalonate}) + \text{H}_2\text{O} = (\text{OH})\text{CCH}_3(\text{CH}(\text{OH})(\text{COOH})_2(\text{citramalate})$ | 30.05                    | 39.29                    |
| $\text{OH})(\text{CCH}_3(\text{CH}(\text{OH})(\text{COOH})_2(\text{citramalate}) + \text{H}_2\text{O} = \text{CH}_3(\text{CO})(\text{COOH}(\text{pyruvate}) + \text{CH}_3\text{COOH}(\text{acetate}$ | 19.35                    | 1.73                     |
| $2\text{CO}_2 + 2\text{H}_2 = \text{OCHCOOH}(\text{glyoxylate}) + \text{H}_2\text{O}$ | 31.73                    | 69.88                    |
| PP cycle                                              |                          |                          |
| $\text{CH}_3(\text{CO})(\text{COOH}(\text{pyruvate}) + \text{H}_3\text{PO}_4 = \text{C}_3\text{H}_5\text{O}_6\text{P}(\text{phosphoenolpyruvate}) + \text{H}_2\text{O}$ | 875.73                   | 917.27                   |
| $\text{CH}_2(\text{CO})(\text{COOH})_2(\text{oxaloacetate}) + \text{H}_3\text{PO}_4 = \text{C}_3\text{H}_5\text{O}_6\text{P} + \text{H}_2\text{O} + \text{CO}_2$ | 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-
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 Ib) 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, \( \Delta G^0_{298} = -629.17 \); \( \Delta G^0_{473} = -592.04 \) 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, \( \Delta G^0_{298} = -30.54 \); \( \Delta G^0_{473} = -35.34 \) 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 = C₃H₆O₄ (2-methylsuccinate) satisfies the energy requirements for autotrophic growth (Thauer and Shima 2008; Beasley and Nunny, 2012; Averesch 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-
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 components 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, 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 ($ΔG^0_{298} = 96.57$ 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 C₄H₄O₄ (fumarate) + CH₄ + [O] = C₂H₄O₂ (acetate) + C₃H₄O₃ (pyruvate), where [O] is an inorganic oxidant. The free energy of reactions with the

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.
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 $\text{C}_4\text{H}_6\text{O}_5 (\text{malate}) + 1.5\text{C}_2\text{H}_4 + 2.5\text{CO}_2 = 2\text{C}_4\text{H}_6\text{O}_5 (\text{two malates})$. 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 twocomponent system (C and H are extensive parameters), since oxygen, represented by the logarithm of the $\text{O}_2$ 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 (associations) 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 $\text{CO}_2$ facies and in the $\text{CH}_4$ facies; i.e., they can develop systems for carbon fixation assimilable in the form of $\text{CO}_2$ or $\text{CH}_4$. 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–$\text{H}_2\text{O}$) and high-temperature (Fum–$\text{H}_2\text{O}$) paragenesis in the $\text{CO}_2$ facies (I) or the formation of low-temperature (Suc–$\text{CH}_4$) and high temperature (Fum–$\text{CH}_4$) paragenesis in the $\text{CH}_4$ (II) facies. Thus, the methane facies (II) is a wide range of thermodynamic stability of systems for the assimilation of $\text{CH}_4$ 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 $\text{CO}_2$ (facies I) but pyrite–pyrrhotite–magnetite (PPM) and quartz–magnetite–fayalite (QMF) equilibria in facies II (methane stability). The last two

Table 2. Free energies of the reactions of the MF branch (I) of the bicycle and the total reaction of $\text{CH}_4$ fixation with the participation of oxidized forms of nitrogen and iron as oxidants under hydrothermal conditions at temperatures of 298 and 473 K and $P_{\text{SAT}}$

| MF cycle reactions | $\Delta G^{0}_{298}$, kJ | $\Delta G^{0}_{473}$, kJ |
|--------------------|--------------------------|--------------------------|
| (CH$_2$)(COOH)$_2$(fumarate) + CH$_4$ = (CH$_2$(CH$_2$CH)(COOH)$_2$(methyl succinate) | -44.95 | -29.97 |
| (CH$_2$)(CH$_2$CH)(COOH)$_2$(methyl succinate) + H$_2$O = (CH$_2$CHCH(OH)(COOH)$_2$(citramalate) + H$_2$ | 96.57 | 94.14 |
| (CH$_3$=CH)(COOH)$_2$(mesaconate) + H$_2$ = (CH$_2$)(CH$_2$CH)(COOH)$_2$(methyl succinate) | -66.53 | -54.85 |
| (CH$_3$=CH)(COOH)$_2$(mesaconate) + H$_2$O = (CH$_2$CHCH(OH)(COOH)$_2$(citramalate) | 30.05 | 39.29 |
| (CH$_2$CHCH(OH))(COOH)$_2$(citramalate) = C$_2$H$_4$O$_2$(acetate) + C$_3$H$_4$O$_3$(pyruvate) | 19.35 | 1.73 |
| **Total fixation of methane** | | |
| C$_4$H$_6$O$_4$(fumarate) + CH$_4$ + H$_2$O = C$_2$H$_4$O$_2$(acetate) + C$_3$H$_4$O$_3$(pyruvate) + H$_2$ | 70.97 | 65.9 |
| C$_4$H$_4$O$_4$ + CH$_4$ + 0.5O$_2$ = C$_2$H$_4$O$_2$ + C$_3$H$_4$O$_3$ | -192.22 | -182.53 |
| C$_4$H$_4$O$_4$ + CH$_4$ + 2HNO$_2$ = C$_2$H$_4$O$_2$ + C$_3$H$_4$O$_3$ + H$_2$O + 2NO | -115.77 | -143.28 |
| C$_4$H$_4$O$_4$ + CH$_4$ + 2NO = C$_2$H$_4$O$_2$ + C$_3$H$_4$O$_3$ + N$_2$O | -274.7 | -250.83 |
| C$_4$H$_4$O$_4$ + CH$_4$ + NO = C$_2$H$_4$O$_2$ + C$_3$H$_4$O$_3$ + 0.5N$_2$ | -276.93 | -264.48 |
| C$_4$H$_4$O$_4$ + CH$_4$ + Fe$_2$O$_4$ + 1.5SiO$_2$ = C$_2$H$_4$O$_2$ + C$_3$H$_4$O$_3$ + 1.5Fe$_2$SiO$_4$ | 45.08 | 25.21 |
| C$_4$H$_4$O$_4$ + CH$_4$ + 3Fe$_2$O$_3$ = C$_2$H$_4$O$_2$ + C$_3$H$_4$O$_3$ + 2Fe$_2$O$_4$ | 22.38 | 13.87 |
| C$_4$H$_4$O$_4$ + CH$_4$ + 0.75FeS$_2$ + 0.25Fe$_3$O$_4$ = C$_2$H$_4$O$_2$ + C$_3$H$_4$O$_3$ + 1.5FeS | 38.78 | 31.54 |

Free energies of aqueous substances formation according to (Amend and Shock, 2001; Marakushev and Belonogova, 2013 (Suppl. Mat.)).
Equilibria determine the temperature redox conditions for the fundamental equilibrium $2\text{Suc} + 2\text{CH}_4 + \text{O}_2 = 5\text{Acet}$, as the basis of methanotrophic acetogenesis. The magnetite facies ($\text{Fe}_3\text{O}_4$) covers the $\text{CH}_4 \leftrightarrow \text{CO}_2$ equilibrium practically over the entire temperature range of the hydrothermal system under consideration. Obviously, the $\text{CO}_2 \leftrightarrow \text{CH}_4$ equilibrium should have developed above the $\text{CO}_2 \leftrightarrow \text{CH}_4$ equilibrium, while the $\text{CH}_4$ fixation systems should have developed below it. Apparently, the simultaneous fixation of $\text{CO}_2$ and $\text{CH}_4$ with the formation of acetate (carboxy-methanotrophy) (Fig. 3) occurred in the magnetite facies ($\text{Fe}_3\text{O}_4$), 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 ($\log(\text{O}_2)$) periodically changed relative to the equilibrium of the fayalite–magnetite–quartz buffer ($\Delta QMF$). For example, in zircons with an age of 3.8 Ga, $\Delta QMF$ ranged from $-6.0$ to $+5.5$, and this redox range is shown in the diagram (Fig. 4). This space completely encompasses both the $\text{CH}_4/\text{CO}_2$ 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 realization autotrophic carbon assimilation. Individual modules of metabolic systems created by ancestors, 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-A19-11907190045-0.

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Translated by P. Kuchina