A Clue for Prebiotic Conversion of Carboxylic Acids and Its Evolutionary Relevance to the TCA Cycle

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(Abstract)

A possibility for the prebiotic conversion of carboxylic acids and the evolutionary onset of a primitive metabolism, which may have served as a medium for the presentday metabolic pathway equipped with enzymes, was examined under submarine hydrothermal environments. We used a flow-reactor system simulating submarine hydrothermal environments for the purpose. It was found that malate, which is one of the TCA cycle member molecules, was synthesized from three other kinds of the member molecules; namely alpha ketoglutarate, succinate and fumarate, in the absence of the relevant enzymes. Furthermore, citrate was synthesized in the solution including pyruvate and all kinds of the TCA cycle member molecules. The synthesis of citrate was significantly suppressed when neither pyruvate nor one of the member molecules was present in the initial setup of the starting reaction solution. These results suggested that submarine hydrothermal environments could have played an important role for the prebiotic conversion and synthesis of carboxylic acids and for the ignition of a primitive metabolism.

Introduction

Submarine hydrothermal vents and their neighborhood have been suggested to be a likely locale for prebiotic synthesis of organic molecules necessary for the emergence of life on the primitive earth [1]-[3]. In fact, the synthesis of various organic molecules has been reported to proceed under simulated hydrothermal environments [4]-[7], though with some inevitable caution on their thermal decomposition to the contrary [8]-[10]. In the similar vein, acetic and formic acids were synthesized from carbon dioxide and water in the presence of metallic ions as catalyst at about 1000 ° C [11]. Furthermore, fatty acids were made from oxalic and formic acids by Fischer-Tropsch type reactions under simulated hydrothermal environment [12]. We then attempted to look for a possibility of prebiotic synthesis of carboxylic acids under submarine hydrothermal environments in order to examine whether hydrothermal environments could also play a positive role for forming a primitive metabolic cycle. Then we chose the TCA cycle as one likely candidate for the most primitive and simplest metabolic cycle (Figure 1). For this purpose, we attempted a series of experiments while employing the TCA cycle members as the initial reactants.

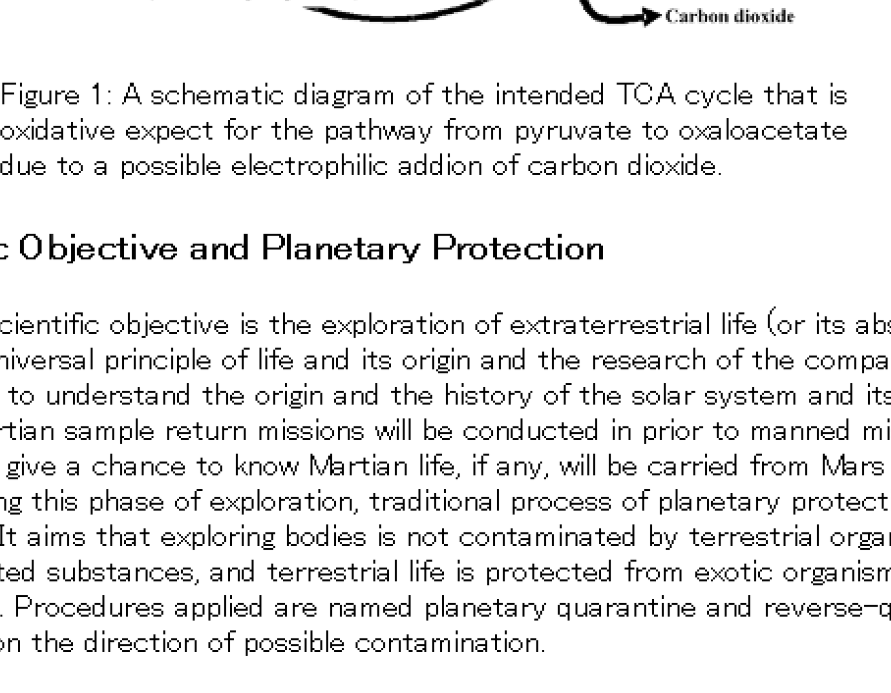

Figure 1: A schematic diagram of the intended TCA cycle that is oxidative expect for the pathway from pyruvate to oxaloacetate due to a possible electrophilic addion of carbon dioxide.

Scientific Objective and Planetary Protection

Major scientific objective is the exploration of extraterrestrial life (or its absence) to know the universal principle of life and its origin and the research of the comparative planetology to understand the origin and the history of the solar system and its planets. In case Martian sample return missions will be conducted in prior to manned mission to Mars, it will give a chance to know Martian life, if any, will be carried from Mars to the Earth. During this phase of exploration, traditional process of planetary protection could be applied. It aims that exploring bodies is not contaminated by terrestrial organisms and life associated substances, and terrestrial life is protected from exotic organisms or substances. Procedures applied are named planetary quarantine and reverse-quarantine depending on the direction of possible contamination.

Materials and Methods

To investigate the reaction of organic acids, we used the flow-reactor system simulating submarine hydrothermal environments. An illustration of this apparatus is displayed in Figure 2 [13]. The starting-solution was prepared by simply dissolving the relevant reactants in pure water. Accordingly, the pH value was not controlled externally. The reactants and products were purchased from Sigma-Aldrich and WAKO, and were identified with the aid of RP-HPLC and NMR equipments. Our HPLC analysis was done with use of both YMC Hydrosphere C18 and YMC Pack-Pro C18 column, 100 mM ammonium phosphate at pH=2.75, and UV at 210 nm. These conditions were found to be functional in detecting all kinds of TCA member molecules; citrate, isocitrate, alpha ketoglutarate, succinate, fumarate, malate and oxaloacetate. A sample profile of their detections is displayed in Figure 3. The NMR analysis was performed with deuterium oxide as solvent. The signal from methyl proton of acetonitrile was designated to be 1.98 ppm as an internal reference. Moreover, NMR samples were purified by HPLC before their analysis.

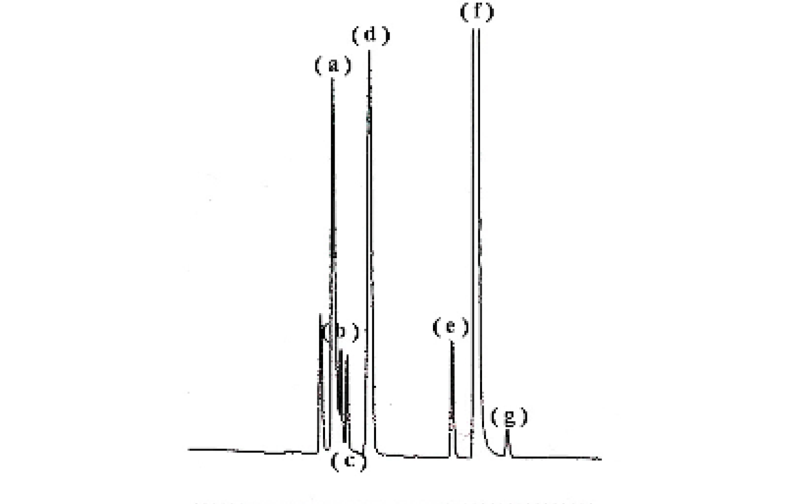

Figure 2: Illustration of a flow-reactor system simulating submarine hydrothermal environments.

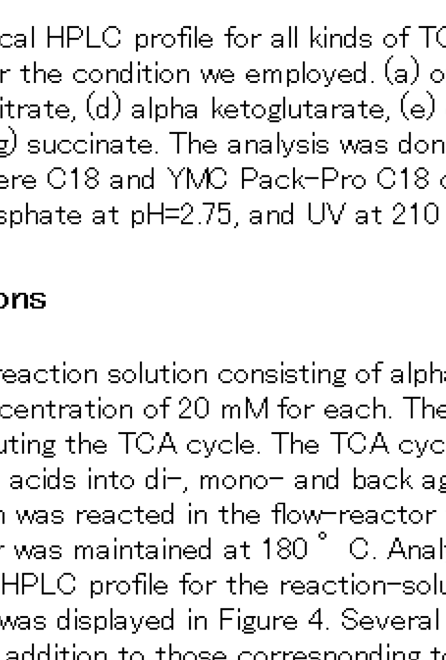

Figure 3: A typical HPLC profile for all kinds of TCA member molecules under the condition we employed. (a) oxaloacetate, (b) malate, (c) isocitrate, (d) alpha ketoglutarate, (e) citrate, (f) fumarate and (g) succinate. The analysis was done with use of YMC Hydrosphere C18 and YMC Pack-Pro C18 column, 100 mM ammonium phosphate at pH=2.75, and UV at 210 nm.

Results and Discussions

We first prepared the reaction solution consisting of alpha ketoglutarate, succinate and fumarate with the concentration of 20 mM for each. These molecules are the member molecules constituting the TCA cycle. The TCA cycle is a reaction cycle transforming tri-carboxylic acids into di-, mono- and back again into tri-carboxylic ones (c.f., Figure 1). This solution was reacted in the flow-reactor for 180 minutes, in which the high-temperature chamber was maintained at 180 ° C. Analysis was done with use of both HPLC and NMR. The HPLC profile for the reaction-solution at 180 minutes after the start of the operation was displayed in Figure 4. Several spectral peaks from the products were detected in addition to those corresponding to the reactants; namely alpha ketoglutarate, succinate and fumarate. As a matter of fact, one of the products was identified to be malate. No isocitrate was identified. The NMR profiles of the products were also displayed in Figure 5. The NMR analysis was performed with deuterium oxide as solvent and with use of the signal at 1.98 ppm due to methyl proton of acetonitrile as an internal reference. In addition, NMR samples were purified by HPLC before their analysis.

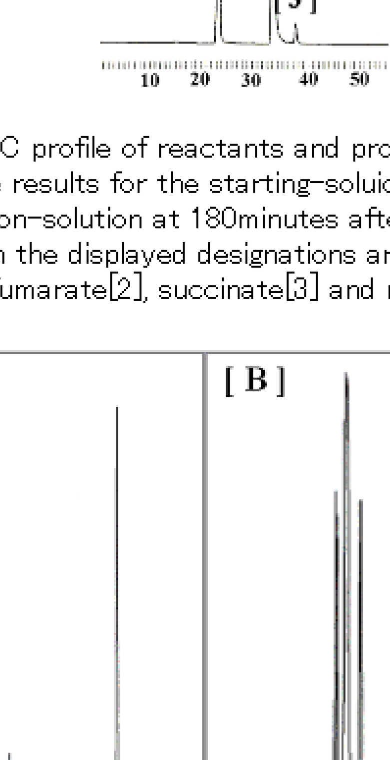

Figure 4: The HPLC profile of reactants and products. The lower pattern shows the results for the starting-soluion and the upper one for the reaction-solution at 180minutes after the start of the operation, in which the displayed designations are alpha ketoglutarate[1], fumarate[2], succinate[3] and malate[RT=20.11].

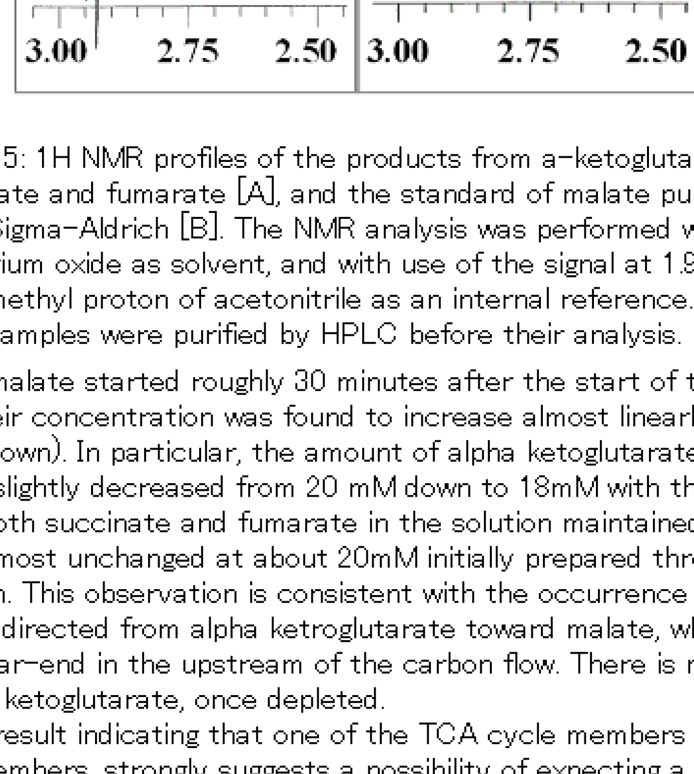

Figure 5: 1H NMR profiles of the products from a-ketoglutarate, succinate and fumarate [A], and the standard of malate purchased from Sigma-Aldrich [B]. The NMR analysis was performed with deuterium oxide as solvent, and with use of the signal at 1.98 ppm from methyl proton of acetonitrile as an internal reference. These NMR samples were purified by HPLC before their analysis.

Synthesis of malate started roughly 30 minutes after the start of the reactor operation, and their concentration was found to increase almost linearly in time since then (data, not shown). In particular, the amount of alpha ketoglutarate present in the reaction solution slightly decreased from 20 mM down to 18mM with the reactor operation, while both succinate and fumarate in the solution maintained their concentrations almost unchanged at about 20mM initially prepared through the entire course of reaction. This observation is consistent with the occurrence of the oxidative reaction pathway directed from alpha ketroglutarate toward malate, while leaving alpha ketoglutarate at far-end in the upstream of the carbon flow. There is no likelihood of replenishing alpha ketoglutarate, once depleted.

The present result indicating that one of the TCA cycle members was synthesized from the other members, strongly suggests a possibility of expecting a primitive reaction cycle out of the available carboxylic acids in hydrothermal environments even in the absence of enzymes of biological origin.

In order to examine a likelihood of having a reaction cycle out of carboxylic acids, we next prepared a reaction solution including all of the TCA cycle member molecules with the concentration of 1.0 mM for each of citrate, is° Citrate, alpha ketoglutarate, succinate, fumarate, malate and oxaloacetate, and 10 mM pyruvate serving as both energy and carbon sources. The temperature of the high-temperature chamber was maintained at 120 ° C. We observed a significant enhancement of the synthesis of citrate over the time interval from the first 40 till 60 minutes after the start of the reactor operation. A time development of citrate is demonstrated in Figure 6.

To examine whether pyruvate may really serve as a carbon source toward the reaction pathway round the cycle, we then ran the reactor for the reaction solution that was different from the previous full solution in that only pyruvate was absent. We also attempted a reaction solution differing from the full solution only in that alpha ketoglutarate was absent. The results are displayed in Figure 6, demonstrating that the synthesis of citrate was significantly disturbed in the absence of any one of the TCA cycle member molecules, e.g., whether pyruvate or alpha ketoglutarate was absent in the initial reaction solution. These findings were in accord with the perspective that a proper operation of the reaction cycle requires the presence of any one of the member molecules constituting the cycle. In fact, when pyruvate in the full reaction solution became depleted with the operation as seen in Figure 6, the synthesis of citrate was also disturbed. The observation that the concentrations of carboxylic acid molecules other than citrate remained rather unchanged through the whole course of the reaction now comes to suggest that there could occure a constant synthesis of each carboxylic acid molecule participating in the cycle while constantly undergoing its conversion into the nearest neighbor molecule in the cycle in a continuous manner. As a matter of fact, the limited enhancement of the concentration of citrate may be due to a slight increase of the rate of citrate synthesis from oxaloacetate relative to the rate of isocitrate synthesis from citrate.

Furthermore, the reaction cycle was implicated to be oxidative in demonstrating the synthesis of malate from alpha ketoglutarate, succinate and fumarate (cf. Figure 5) and no likelihood for synthesizing isocitrate. Indirect evidence on the oxidative cycle, instead of the reductive one, came from the measurement of the pH value of the reaction solution. The pH value increased with the reactor operation, though only slightly [14]. Increase in the pH value suggested that water molecules in the aqueous solution could function as an oxidant as accepting electrons from reactants and products through their dehydration and decarboxylation [15].

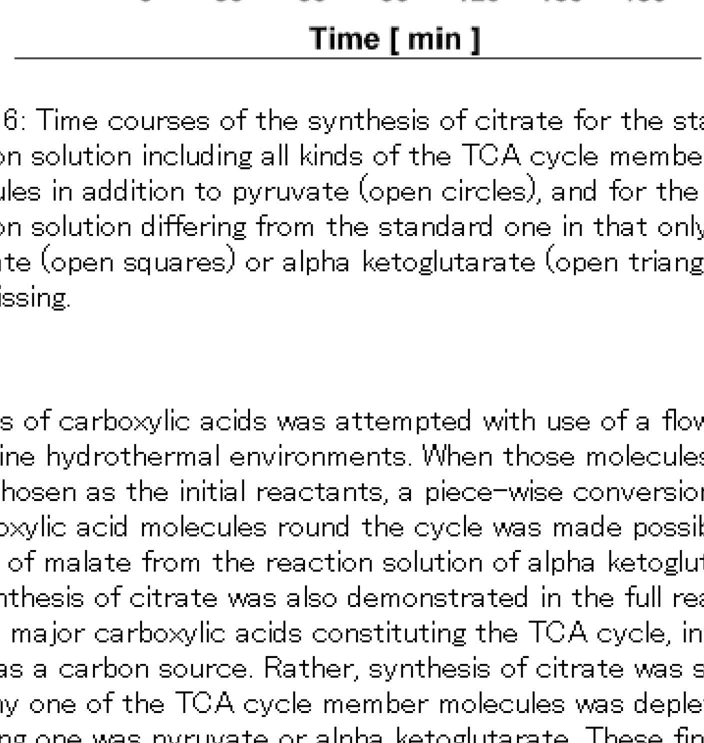

Figure 6: Time courses of the synthesis of citrate for the standard reaction solution including all kinds of the TCA cycle member molecules in addition to pyruvate (open circles), and for the reaction solution differing from the standard one in that only pyruvate (open squares) or alpha ketoglutarate (open triangles) was missing.

Conclusion

Prebiotic synthesis of carboxylic acids was attempted with use of a flow-reactor simulating submarine hydrothermal environments. When those molecules constituting the TCA cycle were chosen as the initial reactants, a piece-wise conversion of the participating carboxylic acid molecules round the cycle was made possible. An example was the synthesis of malate from the reaction solution of alpha ketoglutarate, succinate and fumarate. Synthesis of citrate was also demonstrated in the full reaction solution including all of the major carboxylic acids constituting the TCA cycle, in addition to pyruvate serving as a carbon source. Rather, synthesis of citrate was significantly disturbed when any one of the TCA cycle member molecules was depleted initially, whether the missing one was pyruvate or alpha ketoglutarate. These findings suggest that submarine hydrothermal environments on the primitive earth might have functioned as reaction-sites for prebiotic carboxylation and for cyclic transformation of carboxylic acids among the participating mono-, di- and tri-carboxylic acid molecules. Once a reaction cycle out of carboxylic acid molecules set in motion, it could also have served as a most primitive metabolic cycle even in the absence of enzymes of biological origin. The reaction cycle we demonstrated may have functioned as an evolutionary precursor of the TCA cycle equipped with enzymes.

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