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Synthesis of α-amino and α-hydroxy acids under volcanic conditions: implications for the origin of life

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ABSTRACT

Facile synthesis of α-hydroxy and α-amino acids is observed at temperatures from 145 to 280 °C with catalytic Ni^{2+}, with cyano ligands as source for C and N, and with CO as a reductant and as a source for C. Implications for the problem of the origin of life are discussed.

While temperature is the most pervasive physical parameter of life, the thermal conditions of the origin and early evolution of life remain unresolved. There are two main theories for the origin of life on Earth: the prebiotic broth theory of a cold, oceanic, heterotrophic origin and the pioneer metabolic theory of a hot, volcanic, chemo-autotrophic origin.[1] Very low temperatures are considered mandatory for the slow accumulation of a prebiotic broth for chemical stability reasons, while the high-temperature synthesis of amino acids under volcanic/hydrothermal conditions is claimed to be too inefficient in an aqueous environment to compete with thermal decomposition.[2,3]

In the context of a hot volcanic origin, the aqueous reaction system $Ni(OH)_2$/KCN/CO under alkaline aqueous conditions was previously shown[4] to produce α-amino and α-hydroxy acids in the moderate temperature range of 100 ± 20 °C, which defines the habitats of hyperthermophilic organisms. We now report an increase of production efficiency by up to an order of magnitude, if the reaction temperature is increased to the ultra-hyperthermophilic range of 145–280 °C with an optimum around 160–180 °C. The increased reaction temperature also allowed the detection of amides as reaction intermediates.

We modeled volcanic/hydrothermal conditions by a low-pressure, heterogeneous batch reaction system combining an aqueous suspension of $Ca(OH)_2$ as buffer in 10 mL water with 2 mmol $NiSO_4$ as a source for catalytic nickel centers, 2 mmol KCN (variously labeled) for providing cyano ligands as a source for N and as a major source for C, and with a CO gas phase as a reductant and as a minor source for C. In comparative experiments, $NiSO_4$ was replaced by $FeSO_4$ or $CoSO_4$. Table 1 lists the variations of reaction conditions and the three major families of organic products. In a typical run, a 50 mL stainless steel laboratory autoclave (Roth) with glass insert was charged with 0.5 g (6.75 mmol) of $Ca(OH)_2$, 524 mg (2 mmol) of $NiSO_4{\cdot}6H_2O$, 132 mg (2 mmol) of KCN, 10 mL of deaerated and Ar-saturated water, and a gas phase of CO (CO 2.5 Air Liquide). The gas pressure at room temperature was chosen so that the total pressure given in Table 1 was reached at the stated reaction temperature. The pH of the reaction mixture was measured at the end of the stated reaction time. The reaction mixture was centrifuged and the supernatant was neutralized with 5 M HCl and freeze-dried. The residue was derivatized with *N*-(*tert*-butyldimethyl-silyl)-*N*-methyl-trifluoroacetamide (TBDMS) in acetonitrile (1 h at 80 °C) for analysis by GC–MS [Shimadzu GC-17A and QP-5000; J&W Scientific DB-5 MS column (length: 30 m, I.D. 0.25 mm, film: 0.25 μm); temperature program and settings: 0–3 min at 90 °C, 3–22 min at 90–280 °C, 10 °C/min; 22–25 min at 280 °C; injector temperature: 260 °C; detector temperature: 260 °C; column flow rate: 1 ml/min; scan interval: 0.5 s; detector voltage: 1.5 kV]. External standards were used for quantitation with subtraction of small concentrations of ^{12}C-lactate detected in blind tests. Glyceramide has been synthesized[5] and used for identification, but not for quantitation because of isolation

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Table 1
Products of carbon fixation reactions under ultra-hyperthermophilic conditions

Run	1	2	3	4	5	6	7	8	9	10	11
Temperature (°C)	100	145	160	160	160	160	180	200	280	160	160
Reaction time (h)	20	20	5	10	20	40	20	20	5	20	20
KCN 2 mmol	$^{12}C^{14}N$	$^{12}C^{14}N$	$^{12}C^{14}N$	$^{13}C^{14}N$	$^{12}C^{15}N$	$^{13}C^{14}N$	$^{13}C^{14}N$	$^{12}C^{14}N$	$^{13}C^{14}N$	$^{13}C^{14}N$	$^{13}C^{14}N$
*p*CO at rt (bar)	65	60	55	55	55	55	50	40	30	7	1
Total pressure [a] (bar)	75	75	75	75	75	75	75	75	80	15	5
$Ca(OH)_2$ g/10 mL	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	1	0.5	0.5
Final pH	12.7	12.8	12.9	12.8	12.6	9.1	12.6	6.5	12.8	12.7	12.9
Products						μmol/10 mL (% $^{13}C_t$)[b]					
*g*1 Glycolate	0.24	2.50	1.70	4.30	6.00	6.30 (92)	4.88	4.40	2.70	2.60	0.63
*g*2 Glycolamide	0.01	0.14	0.54	3.70	0.23	0.02	0.02	0.03	—	—	—
*g*3 Glycine	0.65	3.40	1.20	3.90	7.80	8.40 (97.5)	6.10	3.40	0.24	2.90	0.91
*g*4 Glycinamide	—	0.15	0.43	1.30	0.12	—	tr	—	—	—	—
$G = 2 \cdot \sum g/20$[c]	**0.09**	**0.62**	**0.37**	**1.32**	**1.42**	**1.47**	**1.10**	**0.78**	**0.29**	**0.55**	**0.15**
*a*1 Lactate	0.08	0.21	0.03	0.23	0.55	0.80 (84)	0.46	0.37	0.30	0.11	0.06
*a*2 Lactamide	—	0.02	0.01	0.13	0.02	—	—	—	—	—	—
*a*3 Alanine	—	0.24	0.03	0.16	0.86	1.00 (91)	0.84	0.50	0.03	0.11	0.03
*a*4 Alaninamide	—	0.02	0.01	0.13	0.01	—	—	—	—	—	—
$A = 3 \cdot \sum a/20$	**0.01**	**0.07**	**0.01**	**0.1**	**0.22**	**0.27**	**0.2**	**0.13**	**0.05**	**0.03**	**0.01**
*s*1 Glycerate	—	0.75	0.49	1.30	2.90	3.50 (98)	1.90	0.71	tr	0.35	—
*s*2 Glyceramide	—	tr	0.50	0.38	0.02	—	—	—	—	—	—
*s*3 Serine	—	0.07	0.05	0.07	0.33	0.21 (94)	0.12	—	—	—	—
*s*4 Isoserine	—	0.07	0.03	0.07	0.47	0.16 (96)	0.19	—	—	—	—
$S = 3 \cdot \sum s/20$	**—**	**0.13**	**0.16**	**0.27**	**0.56**	**0.58**	**0.33**	**0.11**	**—**	**0.05**	**—**

[a] $p(H_2O + CO)$ at reaction temperature; tr 0.002–0.005 μmol/10 mL; — <0.002 μmol/10 mL.
[b] % $^{13}C_t$ in run 6 = % totally ^{13}C-labeled isotopomer ($^{13}C_2$ or $^{13}C_3$).
[c] $\sum g = g1 + g2 + g3 + g4$; $\sum a = a1 + a2 + a3 + a4$; $\sum s = s1 + s2 + s3 + s4$; *G*, *A*, *S* = % cyanide-C entering pathways to Gly, Ala, Ser product family.

difficulties. The quantity of glyceramide was estimated by assuming the same peak intensity ratio for glycerate:glyceramide as for lactate:lactamide.

Table 1 lists the three main organic product families: G = Gly-family (glycolate, glycolamide, glycine, glycinamide); A = Ala-family (lactate, lactamide, alanine, alaninamide); S = Ser-family (glycerate, glyceramide, serine, isoserine). The yields in μmol are stated as concentrations in the 10 mL water phase of the reaction system. For the representative example of run 6, the ratios of totally ^{13}C-labeled products to the sums of all isotopologues [$^{13}C_2/(^{13}C_2 + {}^{13}C^{12}C + {}^{12}C_2)$ for C_2-products and $^{13}C_3/(^{13}C_3 + {}^{13}C_2{}^{12}C + {}^{13}C^{12}C_2 + {}^{12}C_3)$ for C_3-products] have been determined by SIM mode and stated as % values. It is apparent that the cyano ligands are the main C-source for the carbon skeletons of the products. The global yield for each product family relative to the cyano carbon atoms is stated, whereby the minor contribution by CO as C-source is not subtracted. The reaction rates depend on the activities of dissolved CO, which are quite low at the high reaction temperatures and at the pressures that were chosen for technical reasons. In case of geologically more realistic higher pressures, the reaction rates are expected to be significantly higher.[6] In repeats of run 5 with Co (or Fe) instead of Ni, we obtained 0.7 (or 0.04) μmol/10 mL glycolate, 0.2 (or 0.06) μmol/10 mL glycine, and traces of lactate and alanine.

We plotted the temperature dependence of the sum of all α-hydroxy acid yields and of the sum of all α-amino acid yields of runs 1, 2, 5, 7, 8 (Fig. 1). The striking similarity of the overall shapes of the two graphs, both with maxima between 160 °C and 180 °C signifies a closely interconnected system of competitive pathways. The steep drop of the yield beyond 160 °C is mainly attributed to the decrease of the ratio of the partial pressure of CO to the partial pressure of H_2O. The reactions are amazingly specific considering the high reaction temperatures, which may be due to the ligand nature of the source for C and N. It is remarkable that the yield increase with increasing temperature is sufficient to overcompensate the yield decrease with decreasing CO pressure (run 1 vs runs 10 and 11).

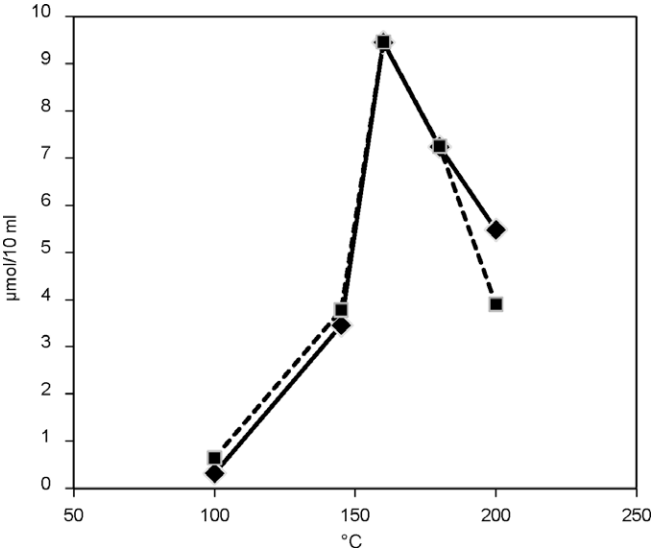

Figure 1. Temperature dependence of the global yields of α-hydroxy acids: glycolate + lactate + glycerate (dashed line) and of α-amino acids: glycine + alanine + serine + isoserine (solid line) in runs 1, 2, 5, 7, 8.

Variation of the reaction time from 5 h to 40 h (runs 3–6) shows that the concentrations of all α-hydroxy and α-amino acids increase throughout the 40 h reaction time, with the exception of serine and isoserine, which drop after 20 h. This reflects the stability of the α-hydroxy and α-amino acids under our reaction conditions. Even serine and isoserine are surprisingly stable in spite of the β-hydroxy and β-amino groups. Moreover, runs 3–6 show that the amides are formed as intermediates, which yield the free α-hydroxy acids and

α-amino acids by hydrolysis. This means that the amides are available as activated products for further metabolic reactions in competition with hydrolysis. In some runs, the increased productivity at higher temperatures allowed the detection of the carbamates of glycine and alanine with CO-derived carbamate groups. Hydantoin as activated derivative of glycine was detected in runs 3 and 4 as double- and triple-silylated derivatives. Hydantoin is structurally related to the imidazole ring of uric acid, a purine. In run 4 the hydantoin was formed as $^{13}C_2$-isotopomer, which indicates that hydantoin forms from glycine amide by ring formation with CO. The mechanism would be analogous to the transformation of a peptide into a peptide derivative with an N-terminal hydantoin ring in the presence of CO and Ni-catalysts as seen in the peptide cycle.[7]

Our results have implications for the problem of the origin of life. They confirm volcanic/hydrothermal sites as efficient sources for α-hydroxy acids and α-amino acids. Moreover, by reflecting a potential hadean chemistry they lend support for a much earlier origin of life than that previously considered. For the context of a heterotrophic origin of life in a prebiotic broth the demonstrated thermal stability of the amino acids relaxes the 'cold ocean' requirement. As to the alternative context of a surface-metabolic autotrophic origin in a volcanic/hydrothermal flow[8] our reactions model the contact between a fluid of liquid water and quenched volcanic gases and a stationary phase of hadean ultramafic rocks. Specifically, our results allow to locate the beginning of organic synthesis in flow zones with temperatures as high as 280 °C. The evolutionary relevance of our reactions is underscored by their analogy with extant biochemistry. Notably, the reducing function of CO is related to the function of anaerobic carbon monoxide dehydrogenase, a nickel enzyme and the reaction products are essential constituents of extant metabolisms.

We anticipate our results to be the starting point for a program of reaction-chromatographic evolution experiments that model volcanic flow conditions by a pressurized flow reactor with a catalytic mineral bed as stationary phase and with pH and temperature gradients along the flow path.[9,10]

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