Aminoacids
Proteinogenic amino acids

genetically encoded

By Matteo Ferla
Biosynthesis of biogenic amino acids

From:
- Pentose phosphate pathway
- Glycolysis
- Citric acid cycle (CAC)

Glucose
- Glucose-6-phosphate (G6P)

4 steps
- Ribose 5-phosphate (R5P)

Histidine (His, H)
- Serine (Ser, S)

Erythrose 4-phosphate (E4P)

3-Phosphoglycerate (3PG)

4 steps
- Phosphoenolpyruvate (PEP)

Phenylalanine (Phe, F)
- Tyrosine (Tyr, Y)
- Tryptophan (Tyr, W)

Pyruvate
- Alanine (Ala, A)
- Valine (Val, V)
- Isoleucine (Ile, I)
- Leucine (Leu, L)

Citrate
- Citric acid cycle (CAC)

Oxaloacetate
- Aspartate (Asp, D)
- Asparagine (Asn, N)
- Methionine (Met, M)
- Threonine (Thr, T)
- Lysine (Lys, K)

3-Ketoglutarate
- Glutamate (Glu, E)
- Glutamine (Gln, Q)
- Proline (Pro, P)
- Arginine (Arg, R)
# Atmosphere composition for young terrestrial planets

<table>
<thead>
<tr>
<th></th>
<th>Reduced</th>
<th>Neutral</th>
<th>Oxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbon (C)</td>
<td>CH₄</td>
<td>CO, CO₂</td>
<td>CO₂</td>
</tr>
<tr>
<td>Nitrogen (N)</td>
<td>NH₃</td>
<td>N₂</td>
<td>N₂</td>
</tr>
<tr>
<td>Oxygen (O)</td>
<td>H₂O</td>
<td>H₂O, CO, CO₂</td>
<td>O₂</td>
</tr>
<tr>
<td>Hydrogen (H)</td>
<td>H₂, CH₄, NH₃, H₂O</td>
<td>H₂O</td>
<td>H₂O</td>
</tr>
</tbody>
</table>
**Miller-Urey experiment - 1952**

Harold Urey (1893-1981)
*UCSD, Nobel prize 1934*
*Discovery of deuterium*

Stanley Miller (1930-2007)
*UCSD San Diego, CA, USA*
Products of the Miller-Urey experiment

Cysteine and methionine also present, when $\text{H}_2\text{S}$ is added to the reaction mixture.
Generation of radicals

High-energy electrons or UV light

\[ \text{H}_2 \rightarrow 2 \text{H}^* \]

\[ \text{H}_2\text{O} \rightarrow \text{H}^* + \text{HO}^- \]

\[ \text{CH}_4 \rightarrow \text{CH}_3^* + \text{H} \]

Radical reactions

\[ \text{CH}_3^* + \text{H}_2\text{O} \rightarrow \text{H}_3\text{C}-\text{O}^- + \text{H}_2 \]

\[ \text{H}_3\text{C}-\text{O}^- + \text{H}^* \rightarrow \text{H}_2\text{C}=-\text{O} + \text{H}_2 \]

\[ \text{H}_3\text{C}-\text{CH}_3 \]

Time (days)

0 1 2 3 4 5 6 7

Ammonia

Amino acids

Hydrogen cyanide

Aldehydes

Ethane
Strecker reaction

Aldehyde + Ammonia $\xrightarrow{\text{heat}}$ Imine

$$\text{R-C-H} + \text{NH}_3 \quad \xrightarrow{\text{heat}} \quad \text{R-C-H}$$

$$\quad \text{R-C-H} \xrightarrow{\text{HCN}} \text{R-C-H} + \text{H}_2\text{O}$$

$$\text{NH}_3 + \text{R-C-C-OH} \xrightarrow{\text{H}_2\text{O}} \text{R-C-C-NH}_2$$

$$\quad \text{R-C-C-NH}_2 \xrightarrow{\text{H}_2\text{O}} \text{R-C-C-CN}$$

Amino acid $\xrightarrow{\text{heat}}$ $\alpha$-Aminocyanonitrile

$$\text{NH}_3 + \text{R-C-C-OH} \xrightarrow{\text{H}_2\text{O}} \text{R-C-C-NH}_2$$

$$\quad \text{R-C-C-NH}_2 \xrightarrow{\text{H}_2\text{O}} \text{R-C-C-CN}$$

Amino acid $\xrightarrow{\text{heat}}$ $\alpha$-Aminocyanonitrile
Scheme 1. Synthesis of $\alpha$-Amino Acids through the Strecker Reaction

$K_1 \gg K_2$

$\text{HO-} \text{CO}_2\text{H} \ \ \text{OH}^- \ \ \text{K}_2$

$\text{HO-} \text{CN} \ \ \text{2} \ \ \longrightarrow \ \ \text{K}_2$

$\text{R}_1 \text{R}_2\text{CN} \ \ \text{R}_1 \text{R}_2\text{R}_2 \ \ \longrightarrow \ \ \text{R}_1 \text{R}_2\text{R}_2 + \text{HCN}$

$\text{R}_1 \text{R}_2\text{CN} \ \ \text{1} \ \ \longrightarrow \ \ \text{K}_1$

$\text{R}_1 \text{R}_2\text{R}_2 + \text{OH}^- \ \ \rightarrow \ \ \text{R}_1 \text{R}_2\text{R}_2 \ \ \rightarrow \ \ \text{K}_1$

$\text{H}_2\text{N-} \text{CO}_2\text{H}$
α-Aminoacid production in the Miller-Urey experiment

TABLE 4.3
Yields of the α-amino acids in the Miller-Urey experiment

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Yield (μM)</th>
<th>Amino Acid</th>
<th>Yield (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>440</td>
<td>Norleucine</td>
<td>6</td>
</tr>
<tr>
<td>Alanine</td>
<td>790</td>
<td>Isoleucine</td>
<td>5</td>
</tr>
<tr>
<td>α-Aminobutyric acid</td>
<td>270</td>
<td>Serine</td>
<td>5</td>
</tr>
<tr>
<td>Norvaline</td>
<td>61</td>
<td>Alloisoleucine</td>
<td>5</td>
</tr>
<tr>
<td>Aspartate</td>
<td>34</td>
<td>Isovaline</td>
<td>5</td>
</tr>
<tr>
<td>α-Aminoisobutyric acid</td>
<td>30</td>
<td>Proline</td>
<td>2</td>
</tr>
<tr>
<td>Valine</td>
<td>20</td>
<td>Threonine</td>
<td>1</td>
</tr>
<tr>
<td>Leucine</td>
<td>11</td>
<td>Allothreonine</td>
<td>1</td>
</tr>
<tr>
<td>Glutamate</td>
<td>8</td>
<td>Tert-Leucine</td>
<td>0.02</td>
</tr>
</tbody>
</table>

Note: Proteogenic amino acids in bold type.
Aminoacid production under hydrothermal conditions

Ni(OH)$_2$/KCN/CO in alkaline aqueous conditions (80-120$^0$C) $\rightarrow$ α-amino and α-hydroxyacids
Huber, C.; Wächtershäuser, G. Science 2006, 314, 630–632

Ca(OH)$_2$/NiSO$_4$/KCN/CO in alkaline (pH 9.1-12.9) aqueous conditions (145-280$^0$C) $\rightarrow$
α-amino and α-hydroxyacids (higher yields): glycine, alanine, serine, glycolate, lactate, glycerate
Extraterrestrial origin of biomolecules

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**Extraterrestrial origin of biomolecules**

Table 1. Soluble Organic Compounds in the Murchison Meteorite

<table>
<thead>
<tr>
<th>class of compounds</th>
<th>parts per million</th>
<th>$n^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>aliphatic hydrocarbons</td>
<td>&gt;35</td>
<td>140</td>
</tr>
<tr>
<td>aromatic hydrocarbons</td>
<td>15–28</td>
<td>87</td>
</tr>
<tr>
<td>polar hydrocarbons</td>
<td>&lt;120</td>
<td>10$^d$</td>
</tr>
<tr>
<td>carboxylic acids</td>
<td>&gt;300</td>
<td>48$^d$</td>
</tr>
<tr>
<td>amino acids</td>
<td>60</td>
<td>75$^d$</td>
</tr>
<tr>
<td>imino acids</td>
<td>nd$^c$</td>
<td>10</td>
</tr>
<tr>
<td>hydroxy acids</td>
<td>15</td>
<td>7</td>
</tr>
<tr>
<td>dicarboxylic acids</td>
<td>&gt;30</td>
<td>17$^d$</td>
</tr>
<tr>
<td>dicarboximides</td>
<td>&gt;50</td>
<td>2</td>
</tr>
<tr>
<td>pyridinecarboxylic acids</td>
<td>&gt;7</td>
<td>7</td>
</tr>
<tr>
<td>sulfonic acids</td>
<td>67</td>
<td>4</td>
</tr>
<tr>
<td>phosphonic acids</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>N-heterocycles</td>
<td>7</td>
<td>31</td>
</tr>
<tr>
<td>amines</td>
<td>13</td>
<td>20$^d$</td>
</tr>
<tr>
<td>amides</td>
<td>nd$^c$</td>
<td>27</td>
</tr>
<tr>
<td>polyols</td>
<td>30</td>
<td>19</td>
</tr>
</tbody>
</table>
Catalytic properties of aminoacids - organocatalysis

Robinson annulation

\[
\begin{align*}
\text{Me} & \quad \text{Me} \\
\text{O} & \quad \text{O} \\
\begin{array}{c}
1 \\
\end{array} & \quad \begin{array}{c}
\text{3 mol\% (S)-proline} \\
\text{DMF} \\
\text{20 °C, 20 h} \\
\end{array} & \quad \begin{array}{c}
\text{2} \\
\text{p-TsOH} \\
\text{PhH} \\
\text{reflux} \\
\end{array} & \quad \begin{array}{c}
\text{3} \\
\end{array}
\end{align*}
\]

100% yield, 93% ee

aldol reaction

\[
\begin{align*}
\text{Me} & \quad \text{R} \\
\text{O} & \quad \text{OH} \\
\text{20 vol\%} & \quad \text{DMSO, r.t.}
\end{align*}
\]

R = H, OH

69% yield
76% ee

97% yield
98% ee

34% yield
72% ee

60% yield
>20:1 dr
>99% ee

Catalytic properties of aminoacids - organocatalysis

**Mannich reaction**

\[
\begin{align*}
\text{Me} & \quad \text{R} \quad + \quad \text{H} & \quad \text{R}' \quad + \quad \text{Me} & \quad \text{O} \\
\text{R} & = \ 	ext{H, CH} & \quad \text{Me} & \quad \text{O} \\
& \quad \text{(solvent or cosolvent)} & \quad \text{(1.1 equiv.)} & \quad \text{O} \\
\end{align*}
\]

\[\xrightarrow{35 \text{ mol\% } (S)-\text{proline}} \]

\[\xrightarrow{\text{r.t.}} \]

\[\xrightarrow{(\text{DMSO or CHCl}_3)} \]

\[
\begin{align*}
\text{Me} & \quad \text{H} \quad \text{N} \quad \text{PMP} \\
\text{Me} & \quad \text{R} \quad \text{N} \quad \text{PMP} \\
\text{Me} & \quad \text{H} \quad \text{N} \quad \text{PMP} \\
\text{Me} & \quad \text{O} \\
\end{align*}
\]

80% yield 93% ee

92% yield >95% de >95% ee

\[
\begin{align*}
\text{MeO} & \quad \text{N} \quad \text{H} \quad \text{Me} \\
\text{Me} & \quad \text{H} \quad \text{N} \quad \text{H} \\
\text{Me} & \quad \text{H} \quad \text{N} \quad \text{H} \\
\text{Me} & \quad \text{H} \\
\end{align*}
\]

\[\xrightarrow{\text{Mannich}} \]

\[
\begin{align*}
\text{MeO} & \quad \text{N} \quad \text{H} \quad \text{Me} \\
\text{Me} & \quad \text{H} \quad \text{N} \quad \text{H} \\
\text{Me} & \quad \text{H} \quad \text{N} \quad \text{H} \\
\text{Me} & \quad \text{H} \\
\end{align*}
\]

\[\xrightarrow{\text{Aldol}} \]

\[
\begin{align*}
\text{MeO} & \quad \text{N} \quad \text{H} \quad \text{Me} \\
\text{Me} & \quad \text{H} \quad \text{N} \quad \text{H} \\
\text{Me} & \quad \text{H} \quad \text{N} \quad \text{H} \\
\text{Me} & \quad \text{H} \\
\end{align*}
\]

**Michael addition**

\[
\begin{align*}
\text{Me} & \quad \text{C} \quad \text{O} \\
\text{Me} & \quad \text{N} \quad \text{O} \\
\text{Me} & \quad \text{N} \quad \text{O} \\
\text{Me} & \quad \text{N} \quad \text{O} \\
\end{align*}
\]

5 mol\% 4

1.5 equiv.

\[
\begin{align*}
\text{Me} & \quad \text{C} \quad \text{O} \\
\text{Me} & \quad \text{N} \quad \text{O} \\
\text{Me} & \quad \text{N} \quad \text{O} \\
\text{Me} & \quad \text{N} \quad \text{O} \\
\end{align*}
\]

91% yield 59% ee

74% yield 66% ee

71% yield 87% ee

\[
\begin{align*}
\text{Me} & \quad \text{O} \\
\text{Me} & \quad \text{O} \\
\text{Me} & \quad \text{O} \\
\text{Me} & \quad \text{O} \\
\end{align*}
\]

3-7 mol\% (S)-proline

20 mol\% cat 5

Catalytic properties of amino acids - organocatalysis

Hydrocyanation

aldehydes

[Chemical structures and reactions]

imines

asymmetric Strecker reaction!!!

The origins of homochirality

Currently known biopolymers are homochiral.
Structural propensity and catalytic activity strongly depends on the enantopurity.
→ Homochirality must have been involved early in the process of life formation.
→ Chiral monomers could be only partially enantioenriched.

General cause of homochirality:
the initial symmetry breaking + subsequent asymmetry amplification:

- The pairity violation
- Stochastic symmetry disturbances

Electroweak interactions and the pairity violation principle cause L-aminoacids and D-sugars to be SLIGHTLY MORE STABLE than their enantiomers.

Differentiation in left and right handedness is inherent property of weak interactions.

Chien-Shiung Wu (1956) – experiment on $^{60}$Co decay.
The origins of homochirality

Circularly polarized light (CPL) from gamma ray bursts

Small enantiomeric excess can be obtained by enantioselective degradation of aminoacids with CPL

Up to 2.6% ee

Stochastic induction of asymmetry – Frank model

If a chiral dissipative structure catalyzes its own formation and inhibits formation of the opposite enantiomer, any stochastic symmetry breaking in the system will be amplified.
autocatalytic Soai reaction – extreme chirality amplification

Organometallic reaction
- NOT prebiotic

Scheme 9. Soai Autocatalytic Reaction

CPL
Aminoacids
$^{12}\text{C}/^{13}\text{C}$-enantiomers!

Extremely sensitive chirality detector
autocatalytic Soai reaction – extreme chirality amplification
autocatalytic Soai reaction – extreme chirality amplification

\[ \text{catalytic, low ee} \rightarrow \text{ZnPr}^+/\text{workup} \rightarrow \text{catalytic, high ee} \]

\[ \text{catalytic cycle} \quad \frac{1}{2} \text{SS} + \frac{1}{2} \text{RR} \quad \frac{1}{2} \text{SS} + \frac{1}{2} \text{RR} \]

\[ S + R \quad \text{catalytic cycle} \quad \text{K}_{\text{dimer}} \]

\[ \text{catalytic cycle} \quad \text{SR} \]

\[ \text{activity of [SR]} \quad \beta = \frac{[\text{SR}]}{[\text{RR}] + [\text{SS}]} \quad K = \frac{([\text{SR}])^2}{([\text{RR}]) ([\text{SS}]}) \]

\[ \text{ee}_{\text{dr}} = \frac{[\text{RR}] - [\text{SS}]}{[\text{RR}] + [\text{SS}] + \gamma [\text{SR}]} \quad \text{ee}_{\text{chiral}} = \frac{\text{ee}_{[2]} + \text{ee}_{\text{chiral}}}{2} \]

\[ \text{7a, } X = \text{Cl,} \quad \text{7b, } X = \text{SCN} \]

\[ (S,S)-8 \quad A = \text{H,} \quad B = \text{Pr}^+ \]

\[ (R,S)-8 \quad A = \text{Pr}^+, \quad B = \text{H} \]

(c)\textsuperscript{19}
autocatalytic organic reactions

Meaningful transformations for the prebiotic syntheses of aminoacids and sugars
**autocatalytic organic reactions**

1 + EtO₂C⁻H + (S)-3 or (R)-3 as catalyst → 3

up to 94% ee

1. (S) or (R) 20-48% yield 85-96% ee
2. without product catalyst 11-36% yield 0.5-9.5% ee

proposed transition-state structure

approach of the enol from the Si face

TS-A

approach of the enol from the Re face

TS-B
Organocatalysis – the origin of homochirality

Table 1. Enantiomeric concentration amplification of phenylalanine after two crystallizations from water

<table>
<thead>
<tr>
<th>Component</th>
<th>Initial ee, %</th>
<th>Final ee, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>10</td>
<td>90.0 ± 3.7</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>91.7 ± 1.5</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>87.2 ± 2.0</td>
</tr>
<tr>
<td>L</td>
<td>10</td>
<td>88.3 ± 1.1</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>88.6 ± 0.9</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>90.9 ± 0.3</td>
</tr>
</tbody>
</table>

Solutions with as little as 1% enantiomeric excess (ee) of D- or L-phenylalanine are amplified to 90% ee (a 95/5 ratio) by two successive evaporations to precipitate the racemate. Such a process on the prebiotic earth could lead to a mechanism by which meteoritic chiral α-alkyl amino acids could form solutions with high ee values that were needed for the beginning of biology.

*Breslow, R., Levine, M. Proc. Natl. Acad. Sci. USA 2006, 103(35), 12979-12980*
Chirality amplification in biphasic systems

Reaction and solution behaviour as a function of the overall proline enantiomeric excess.

a, Product enantiomeric excess versus proline enantiomeric excess for the aldol reaction of equation

b, Solution proline enantiomeric excess (left axis, triangles) and solution proline concentration (right axis, diamonds) as a function of the overall enantiomeric excess for proline at 0.1 M

**Chirality amplification in biphasic systems**

Table 1 | Solution enantiomeric excess at the eutectic point in water at 25 °C for selected amino acids

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>ee of solution at eutectic (%)</th>
<th>Amino acid</th>
<th>ee of solution at eutectic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threonine</td>
<td>0</td>
<td>Methionine</td>
<td>85</td>
</tr>
<tr>
<td>Valine</td>
<td>46</td>
<td>Leucine</td>
<td>87</td>
</tr>
<tr>
<td>Alanine</td>
<td>60</td>
<td>Histidine</td>
<td>93</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>83</td>
<td>Serine</td>
<td>&gt;99</td>
</tr>
</tbody>
</table>

Condensation of amino acids into peptides
Biochemical condensation of amino acids into peptides
Prebiotically relevant peptide condensation agents

<table>
<thead>
<tr>
<th>Entry</th>
<th>Activating agent</th>
<th>Hydrolysis/hydration product</th>
<th>ΔG°/kJ mol⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NH₂CONH₂</td>
<td>CO₂ + NH₃</td>
<td>-16ᵃ</td>
</tr>
<tr>
<td>2</td>
<td>COS (g)</td>
<td>CO₂ + H₂S</td>
<td>-17ᵃ</td>
</tr>
<tr>
<td>3</td>
<td>Pyrophosphate</td>
<td>Phosphate</td>
<td>-19ᵇ</td>
</tr>
<tr>
<td>4</td>
<td>CO (g)</td>
<td>HCO₂H</td>
<td>-16ᵃ</td>
</tr>
<tr>
<td>5</td>
<td>HNCO</td>
<td>CO₂ + NH₃</td>
<td>-54ᵃ</td>
</tr>
<tr>
<td>6</td>
<td>HCN</td>
<td>HCO₂H + NH₃</td>
<td>-75ᵃ</td>
</tr>
<tr>
<td>7</td>
<td>RCN</td>
<td>RCO₂H + NH₃</td>
<td>-80ᶜ</td>
</tr>
<tr>
<td>8</td>
<td>NH₂CN</td>
<td>Isourea</td>
<td>-83ᵈ</td>
</tr>
<tr>
<td>9</td>
<td>HNCNH</td>
<td>Isourea</td>
<td>-97ᵈ</td>
</tr>
<tr>
<td>10</td>
<td>HCCH (g)</td>
<td>CH₃CHO</td>
<td>-112ᵃ</td>
</tr>
</tbody>
</table>

SIPF copper complex geometry with two glycine ligands, optimized by ab initio Hartree–Fock calculations.


Condensation of amino acids into peptides

Scheme 1. Synthesis of \(\alpha\)-Amino Acids through the Strecker Reaction

\[
\begin{align*}
R_1R_2CO_2H & \overset{K_2}{\rightleftharpoons} R_1R_2C{\text{N}} \overset{K_1}{\rightleftharpoons} R_1R_2C{\text{N}} + \text{HCN} \overset{K_2}{\rightleftharpoons} R_1R_2{\text{NH}} - \overset{K_1}{\rightleftharpoons} R_1R_2{\text{CO}_2H}
\end{align*}
\]

Scheme 2. Bücherer–Bergs Hydrolysis of \(\alpha\)-Aminonitriles

\[
\begin{align*}
\text{CO}_2/\text{HCO}_3^- & \rightarrow R_1R_2{\text{CN}} \overset{\text{peptides}}{\rightarrow} R_1R_2{\text{NH}} - \overset{\text{peptides}}{\rightarrow} R_1R_2{\text{CO}_2H}
\end{align*}
\]
**Carbonyl sulfide – condensing agent**

\[ \text{S}=\text{C}=\text{O} + \text{H}_2\text{N}\text{COO}^- \rightarrow \text{NH}_2\text{COO}^- \]

Carbonyl sulfide – condensing agent

Table 2. COS-mediated formation of mixed peptides. Abbreviations for the amino acid residues: A, Ala; F, Phe; L, Leu; S, Ser; Y, Tyr.

<table>
<thead>
<tr>
<th>Entry*</th>
<th>l-Phe (mM)</th>
<th>Reactant 2 (mM)</th>
<th>PbCl₂ (mM)</th>
<th>Final pH</th>
<th>Time (hours)</th>
<th>Observed dipeptides†</th>
<th>Observed tripeptides†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>l-Tyrosine (10)</td>
<td>20</td>
<td>7.2</td>
<td>3</td>
<td>FF, YY, (YF), (FY)</td>
<td>YYY, (YFF), (YFF), FFF</td>
</tr>
<tr>
<td>2</td>
<td>25</td>
<td>l-Leucine (25)</td>
<td>50</td>
<td>7.1</td>
<td>3</td>
<td>FF, LL, (FL)</td>
<td>(LLF), (LFF), FFF</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>l-Alanine (25)</td>
<td>50</td>
<td>5.9</td>
<td>3</td>
<td>FF, (AF)</td>
<td>(AAF), (AFF), FFF</td>
</tr>
<tr>
<td>4</td>
<td>25</td>
<td>l-Serine (25)</td>
<td>50</td>
<td>6.3</td>
<td>3</td>
<td>SS, FF, SF, FS</td>
<td>SSS, (SFF), FFF</td>
</tr>
</tbody>
</table>

*Each experiment was initiated by admitting ~20 ml of COS gas to an argon-purged reaction vessel containing 2 ml of the reaction mixture indicated dissolved in 500 mM Me₃N buffer, at an initial pH of 9.1. Peptide products were identified by LCMS after quenching the reaction at 3 hours.

†Peptides for which product masses were observed but primary amino acid sequences which were not determined are indicated in parentheses.
A slow formation of NCAs from free amino acids and COS in the absence of oxidizing or alkylating agents has been reported and studied through theoretical chemistry investigations. However, it seems unlikely that COS ($\Delta G_0 = 16.9 \text{ kJ/mol}$) could be able to generate NCA ($\Delta G_0 = 60 \text{ kJ/mol}$) in spite of its cyclic structure.

A photochemical activation of thiocarbamate that could take place in a way similar to that of thioacetate in aqueous solution may provide an explanation to this observation. This potential photochemical reaction may also constitute an efficient pathway for the prebiotic formation of NCAs.
Carbonyl sulfide – photochemical activation

Pathways for the formation of NCAs and further reactions including polymerization and interactions with inorganic phosphate (Pi), nucleotides (NMP), and RNA.

Diketopiperazines as intermediates for peptide condensation
Condensation of amino acids into peptides
**Prebiotic peptide condensation in water**

GADV-protein world

α-helix (Ala)

β-sheet (Val)

β-turn (coil) (Gly)

hydrophilic and hydrophobic structures

globular structures

catalytic activity (Asp)
Basic amino acids for primitive genetic code?

Primordial genetic code might have involved only 4 “GNC” codons:
  • **GGC** for glycine
  • **GCC** for alanine
  • **GAC** for aspartic acid
  • **GUC** for valine

Later, the ‘GNC’ code probably evolved into ‘SNS’ code (S = G/C, N = A, U, G, C) – 16 codons encoding 10 basic amino acids (Gly, Ala, Asp, Val, Glu, Leu, Pro, His, Glu, Arg)
**Reduced aminoacid alphabet**

9-aminoacid alphabet is sufficient to construct functional enzymes

Aminoacids: Asp, Glu, Asn, Lys, Phe, Ile, Leu, Met, Arg

**AroQ structure and active site.** A, the homodimeric EcCM is shown with a transition state analog inhibitor bound at its active sites; the two identical polypeptide chains are colored blue and pink for clarity. B, proposed interactions between residues in the evolved active site of the simplified enzyme and the transition state analog inhibitor, compound 1 (red), based on the x-ray structure of EcCM. Residues Gln^{88} and Ser^{84} in EcCM are substituted with Glu^{88} and Asn^{84} in the 9-amino acid enzyme. Residue numbers are referenced to EcCM.

Evolution of a metalloenzyme from short peptides

Zinc-mediated assembly of helix-turn-helix fragments, followed by fusion and asymmetric diversification, afforded MID1sc10, an efficient metalloesterase.

Evolution of a metalloenzyme from short peptides

Crystal structure of MID1sc10
zinc ion - orange sphere,
coordinating histidines - green sticks
linkage of two polypeptides – orange sticks
beneficial mutations - magenta spheres,
residues replaced to prevent competitive zinc
binding modes - cyan spheres).

The evolved variant MID1sc10 is highly enantioselective as a consequence of a 2200-fold specificity switch from the modestly (R)-selective starting catalyst MID1sc.

Michaelis-Menten plots for MID1sc (yellow and inset) and MID1sc10 (green) show a 70,000-fold improvement in hydrolysis efficiency for (S)-configured 1 after optimization.

**Aminoacids - Summary**

Prebiotic generation plausible – variants of the Miller-Urey experiment
Strecker-type of chemistry likely

Aminoacids are good catalysts, can perform various chemical transformations

The origin of homochirality in the Universe caused by the parity violation and stochastic fluctuations

Chirality amplification possible in numerous chemical reactions

Aminoacids can catalyse their own formation with chirality amplification and undergo physical enantioenrichment processes

Condensation of aminoacids into peptides plausible under prebiotic conditions using condensing agents

Simple peptides can exhibit broad structural variety, catalytically active enzymes can be constructed with reduced aminoacid alphabet