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Primordial reductive amination revisited

Claudia Huber[a] and Günter Wächtershäuser[b,*]

[a]*Department for Organic Chemistry and Biochemistry, Technische Universität München, Lichtenbergstraße 4, D-85747 Garching, Germany*

[b]*Tal 29, D-80331 München, Germany*

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Abstract—Amino acids are formed efficiently by reductive amination of α-keto acids under aqueous, conditions with freshly precipitated FeS or $Fe(OH)_2$ and with NH_3, CH_3NH_2 or $(CH_3)_2NH$ at pH values near their pK_a.

Amino acids are essential constituents of the metabolism. It is therefore not surprising that the problem of the primordial sources of amino acids plays a major role in all theories on the origin of life. The theory of a chemo-autotrophic origin of life[1,2] assumes a primordial biosynthesis of amino acids by reductive amination of α-keto acids. It has been reported earlier that such a primordial reaction channel does indeed exist.[3] We here report experiments to study the mechanism of this reaction.

We found that experiments on reductive amination of α-keto acids in the presence of FeS in water are inflicted with a reproducibility problem, which was found to be due to the state of FeS. It was determined that reproducibility can be improved if the reactions are carried out with FeS that has been freshly precipitated from $FeSO_4$ for each reaction.[4] With such freshly and identically prepared FeS, the results were found to be reproducible enough to lead to conclusive comparisons and for carrying out optimization experiments. It is noteworthy that this procedure avoids a dehydration of FeS and therefore it is geo-chemically more relevant than the previously use of dried FeS. Furthermore, we discovered that the reaction is sensitively dependent on the pH of the aqueous reaction medium. Therefore, conclusive comparisons could be made only by paying close attention to the pH value.

Table 1 shows the results of representative experiments on the formation of alanine, glutamic acid, phenylalanine, tyrosine, *N*-methyl-phenylalanine and *N*,*N*-dimethyl-phenylalanine from 100 μmol of the corresponding α-keto acid in the presence of 2 mmol FeS and 15 mmol NH_4Cl, NH_4HCO_3, CH_3NH_2 and $(CH_3)_2NH$, respectively. Column 3 shows the maximum yield of the amino acid at the pH given in parentheses.[5] Column 4 shows yields at selected pH-values above and

Table 1. Yield of various amino acids by reductive amination with various amino sources. The pH for each yield measurement is given in parentheses. The pH-dependence of the yield is indicated for each pair of amino acid and amino source by one lower and one higher yield (pH) measurement (Me-Phe *N*-methyl-phenylalanine; Me_2-Phe *N*,*N*-dimethyl-phenylalanine)

Amino acid	Amino source	Yield max mol% (pH)	Yield range mol% (pH)	Temp. (°C)	Time (h)
Ala	NH_4Cl	52 (8.8)	10 (8.6)–20 (9.3)	50	20
Glu	NH_4Cl	35 (8.9)	25 (8.4)–20 (9.6)	50	72
Phe	NH_4Cl	53 (9.2)	32 (8.5)–14 (10.5)	75	17
Phe	NH_4HCO_3	49 (9.3)	29 (8.6)–38 (10.1)	75	17
Tyr	$(NH_4)_2SO_4$	58 (9.1)	15 (8.3)–34 (9.8)	75	17
Me-Phe	CH_3NH_2	83 (10.1)	51 (7.3)–53 (11)	75	17
Me_2-Phe	$(CH_3)_2NH$	18 (11.1)	4 (9.5)–6 (12.7)	75	17

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* Corresponding author. Tel.: 0049-89-2199760; fax: 0049-89-223759; e-mail: info@patent.de

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below the optimum pH. It appears that the optimum pH is roughly identical with the pK_a of the amino source. For NH_3, optimum pH values from 8.8 to 9.3 correspond to the pK_a of ammonia of 9.25. For CH_3NH_2 an optimum pH value of 10.1, corresponds to the pK_a of methylamine of 10.4. For $(CH_3)_2NH$ an optimum pH value of 11.1 corresponds to the pK_a of dimethylamine of 10.9.

$$R'\text{-CO-COOH}+R_2NH+2H^++2e^-\rightarrow R'\text{-CH}(NR_2)\text{-COOH}+H_2O.$$

No significant difference was detected between the yields when NH_4Cl or NH_4HCO_3 is used as amino source. The previous[3] failure of reductive amination in the presence of NH_4Cl without carbonate is not explainable. It may have been due to a lack of pH-control or due to the state of FeS.

All attempts have failed to convert oxaloacetic acid to aspartic acid with FeS at room temperature (3 days), 50°C (3 days) and 100°C (17 h) in the pH range from 6.4 to 9.8, while it has been shown that aspartic acid is quite stable under these conditions (e.g. 94% remaining after 3 days at 50°C and pH 9.0). This may be due to the predominance of a non-reactive intermediate enamine structure in this case, while in the other cases a reactive imino structure may be assumed to be the dominant species. Instead of aspartic acid, small amounts of alanine (up to 10 mol%) were formed, probably due to decomposition of oxaloacetate to pyruvate.

As shown in Table 2, excess amounts of Na_2S over the stoichiometric amount for 2 mmol $FeSO_4$ lead to a decrease of the yield of amino acids. This may be due to a blockage of catalytic iron sites.

We have also found that the yield of amino acid strongly decreases with increasing ionic strength of the reaction medium. In Table 3, this is shown for *N*-methyl-phenylalanine and pH 10.3 to 10.7. A similar salt effect is found at other pH values and for other amino acids. Such a negative salt effect is explained by an ionic mechanism that involves an interaction of oppositely charged species. It has the practical consequence that the reaction rate can be increased by decreasing the concentration of ions in the aqueous medium. Further, it is an important hitherto hidden parameter for carrying out comparisons.

Table 4 shows the effect of an addition of increasing amounts of air. The reaction is not inhibited by low amounts of air, which are probably consumed by FeS. Only large amounts of air inhibit the reaction.

The temperature dependence of the reductive amination over time is shown in Table 5 for the case of tyrosine. At 100°C the reaction is complete after 1 h. Even at 4°C, the reaction still proceeds, albeit at a very slow rate.

If FeS is replaced by sulfides freshly precipitated from $CoSO_4$, $NiSO_4$, $MnSO_4$, $ZnSO_4$, Ag_2SO_4, $CuSO_4$, $MgSO_4$, $CrCl_3$, no reductive amination is observed. If 50 mol% of the FeS is replaced by NiS the yield of phenylalanine is about 50 mol% of that with FeS alone. If FeS is replaced by freshly precipitated $FeCO_3$ no reductive amination is observed. Surprisingly, however, if FeS is replaced by freshly precipitated $Fe(OH)_2$, efficient reductive amination is observed. In a typical reaction series with NH_4Cl we obtained a maximum yield of 23 mol% Phe at pH 9.3. This maximum yield is about half the maximum yield with FeS. The increased rate in the presence of FeS may be due to a promotion by sulfido ligands or to the mass effect of a removal of ferric ions by the formation of pyrite from FeS and ferric ions, which is well known to be a rapid reaction.[6] Importantly, however, the result with $Fe(OH)_2$ shows that neither the presence of sulfido ligands nor the formation of pyrite are necessary for reductive amination. It may be speculated that the primary oxidation

Table 2. Yield of amino acids in the presence of excess sulfide

Amino acid	Phe	Phe	Tyr
Amino source	NH_4Cl	NH_4HCO_3	NH_4HCO_3
Temp./time	75°C/17 h	75°C/17 h	100°C/40 h
mmol Na_2S	mol% (pH)	mol% (pH)	mol% (pH)
2	53 (9.2)	38 (8.6)	68 (8.8)
3	46 (9.0)	31 (8.6)	58 (8.8)
4	31 (9.3)	21 (8.7)	26 (8.7)

Table 3. Dependence of amino acid yield on the ionic strength

N-Methyl-Phe	
Ionic strength 10^{-4} mol/cm^3	Yield mol%
21	75
24	71
36	64
51	44
81	42

Table 4. Inhibition of reductive amination by air

Air vol%	Phe μmol
0	30
0.9	23
9	10
100	2

Table 5. Dependence of reductive amination on temperature

	Mol% Tyr		
Temp. (°C)	1 h	17 h	40 h
20	9	15	26
50	17	66	70
75	39	66	69
100	87	81	86

products are ferric centers and that the polynuclear colloidal state of freshly precipitated FeS and/or $Fe(OH)_2$ is a necessary requirement for the reaction.

$$2FeS \rightarrow FeS_2 + Fe^{2+} + 2e^-$$

$$2Fe(OH)_2 \rightarrow 2FeO(OH) + 2H^+ + 2e^-.$$

In spite of intense efforts, it was not possible to demonstrate the previously suggested[2] coupling of the reductive amination with an activation of the carboxylic acid group of the amino acids. The carboxylic acid group is apparently not involved in the reaction center. This was confirmed by a set of experiments (data not given) which show that methyl phenyl-glyoxylate undergoes facile reductive amination to the methyl-ester of phenyl-glycine. It has, however, been reported earlier that under very similar conditions amino acids are activated with CO to form peptides.[7]

Our results show that the mechanism of this reaction is more complex than previously appreciated. They open a new avenue of investigation regarding the mechanistic relationship of FeS and $Fe(OH)_2$ in the reductive amination to amino acids and in other reactions[8] of a presumptive primordial metabolism. Thus our results seem to point in the direction of a revision of the theory of a chemo-autotrophic origin of life and thus may bring us closer to the ultimate achievement of the kind of synthetic autocatalytic domino reactions, with which life is perceived as having initiated itself.

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4. All solutions were prepared from doubly distilled water, which was boiled and cooled under a stream of argon. Serum bottles (120 ml) were typically charged with 2 mmol solid $FeSO_4 \cdot 7H_2O$, 15 mmol NH_4 source (as chloride, hydrogen carbonate or sulfate) or alternatively the hydrochloride of CH_3NH_2 and $(CH_3)_2NH$, 100 μmol α-keto acid (as sodium salt), closed with Viton stoppers (Ochs), deaerated and subsequently charged with water and an appropriate amount of aqueous $Na_2S \cdot 9H_2O$ for the in situ precipitation of FeS. Subsequently the serum bottles were charged with appropriate amounts of HCl or NaOH for adjusting the pH. The total liquid volume was brought to 10 ml. Thereafter the reaction was carried out for 17 h at 75°C. All chemicals were purchased from Aldrich.
5. For analysis samples of the liquid reaction mixture were centrifuged and the pH of the supernatant liquid was measured. The yield of the resulting amino acid was determined by HPLC 10C18 column with an H_2O/MeOH gradient (0–100% MeOH) and 0.1% phosphoric acid, using a Merck–Hitachi Pump L-7100 and a Merck–Hitachi UV detector L-7400 at the appropriate wavelength (254 nm for aromatic amino acids and 190 nm for aliphatic amino acids). In addition the yields of the amino acids were confirmed by reaction with *ortho*-phthaldialdehyde and HPLC analysis according to Bober, H. *Beckman Report* **1986**, *12*, 3–5.
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