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Investigating Metal Oxide - Catalysed Thermal Reaction for Prebiotic Peptides Synthesis: A Comparative Study

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Abstract

We conducted an experimental study on the spontaneous formation of peptides from two parallel reactions gly-asp acid/val mixtures in aqueous media and in the presence and absence of metal oxide catalysts, specifically alumina and silica, at 85 ± 5°C under simulated early Earth drying-wetting cycles. Analytical techniques revealed the synthesis of homogeneous and heterogeneous peptides, including cyclic glycine structures, resulting from intra- and intermolecular condensation reactions. Peptide yield was influenced by thermal treatment duration and, to a lesser extent, the presence of metal oxide catalysts.

Notably, the gly-val system preferred to form glycine homo-oligomers up to tetrameric levels and heteropeptides including diketopiperazine. Conversely, the gly-asp acid system displayed limited oligomerization, reaching only trimeric levels, and yielded glycyl-aspartic acid. These findings offer valuable insights into the potential mechanisms underlying protein evolution on primitive Earth.

Keywords: Glycine, Aspartic acid, Valine, Metal oxides, Primitive Conditions

1. Introduction

The beginning of life on Earth has been a long-standing topic of scientific analysis, with various theories proposed to explain the appearance of biomolecules. The abiotic fusion of polynucleotides and proteins has been a research focus, with studies [1-6] investigating the formation and accumulation of these biopolymers under prebiotic conditions. However, the complex pathways involved in peptide formation and evolution on primitive Earth remain unclear. Previous research has suggested that peptides may form through amino acid dimerization, cyclization, sequence inversion, and chain elongation, with decomposition reactions such as hydrolysis, deamination, and decarboxylation also occurring. Many workers have critically discussed the possible role of aluminosilicates, alumina, and silica in the biological chemical evolution of biomolecules [7,8,9] following Bernal [10]. The synthesis of macromolecules such as proteins and nucleic acids is of great importance from the viewpoint of chemical evolution. It has been shown that amino acids polymerise under the influence of heat at elevated temperatures, both in aqueous and in anhydrous conditions. Recently, various adsorption studies [11-13] have established that amino acids and other bio-monomers could easily adsorb on different natural catalysts, silica, and alumina, thereby concentrating on their surfaces in the formation of oligopeptides. Amino acid condensation catalysed by inorganic oxide surfaces is a widely recognised way for prebiotic peptide formation in the planets of a terrestrial group [4,9,14-15]. Moreover, decomposing reactants and products would likely accompany oligomerisation at higher temperatures. After the amino acids abiogenic synthesis, the next step of chemical evolution was probably the polymerization of these monomers to form peptides and polypeptides. The biological significance of proteins and the abiotic synthesis of peptides or polypeptides has received more attention than other biological polymer. Earlier, leading investigators Fox [16] and Harada [17] reported the formation of linear peptides by heating acidic amino acids such as aspartic acid and glutamic acid with sixteen other amino acids at a temperature range of 180-200 and proposed that glutamic acid was more effective in promoting this condensation reaction via lactum formation.

Considering the importance of widely distributed metal ions and metal oxides near seashores, the prebiotic synthesis of peptides has been carried out from thermal energy at a temperature below the boiling point of water. The synthesis of peptides has been carried out from glycine without any alkyl substituent at the α-alpha carbon atom and aspartic acid dicarboxylic acid as the reactant amino acids. The main objectives of this study were to explore the effect of neutral and acidic amino acids on the formation of oligopeptides under wetting drying conditions. This study aims to investigate the impact of heat on the aqueous environment of glycine, aspartic acid, and valine in the presence and absence of silica and alumina. The goal is to explore the feasible synthesis of peptides under drying and wetting conditions, simulating those found near the lithosphere-hydrosphere boundary of primitive seas.

2. Experimental

We used the amino acids and metal oxides in the experimental investigation from Sigma Aldrich Co. Sterilised aqueous solution of the mixture of glycine and aspartic acid, as well as glycine and valine (0.1M each), were taken separately in round bottom borosil glass vessels containing 5ml of each reactant amino acid solution fitted with air condensers kept on the hot plates at the temperature at 85 ± 5°C. Evaporation cycles were performed to simulate a primitive sea beach scenario. The reaction mixture was heated in the presence and absence of metal oxides (0.1g each) for 8-10h in each cycle to achieve complete evaporation. Then 2ml distilled water was added to perform the next evaporation cycle. In every experiment, an identical solution heavily wrapped in several folds of black cloth or paper was kept alongside the reaction

vessels in small Pyrex flasks without heating. Heating was continued for up to 250 h, with analysis performed at 50, 100, 150, 200, and 250 for the possible formation of peptides and the other related amino acids, both by paper chromatography [18] and HPLC [19] methods. Paper chromatography was performed using solvents n-butanol: acetic acid: water (4:1:1 v/v as well as 4:1:5v/v Upper layer) and reagents ninhydrin, isatin while HPLC analysis used a Shimadzu (SPD10A, UV-vis) HPLC apparatus with a C_{18} column and UV detection at 210 nm. The mobile phase was 25% CH_3CN:75% Na_2HPO_4, pH was adjusted to 2.5 with H_3PO_4 at 23-25ºC and the flow rate was monitored at 1.0-1.2 ml/min. Reaction products were identified by Rf values, ninhydrin, HPLC retention time, and UV (Jasco V-550 spectrophotometer) and IR (Perkin-Elmer DX-II FT-IR spectrophotometer) spectra compared to authentic reference substances. Optical density was measured using an MK III Colorimeter monitored at 570 nm, and reaction yields were determined as a percentage of reactants converted to products.

3. Results and Discussion

Two parallel reactions of glycine and aspartic acid as well as glycine and valine in the presence and absence of metal oxides under drying /wetting conditions were investigated for the feasible formation of peptides. Analysis of concentrates of reaction showed the formation of both homo and hetero peptides including Cyclic peptide indicating that intermolecular and intramolecular condensation reactions have occurred. Besides residual reactants, some new amino acids were also formed. In gly-asp and water vapour heated for about 30h the formation of four ninhydrin-positive products on the paper chromatogram. An increase in the number of thermal products was observed and all nine spots formed when the reaction concentrate heated up to 50h was analysed. Out of these, spots corresponding to products IV, VI, and VII were characterized as glycyl-aspartic acid, gly^3, and gly^2, respectively Products III and VIII were identified as residual aspartic acid and glycine whereas lysine (1), DKP (V), and α-alanine (IX) were identified as new thermal products. The identity of product II could not be ascertained. Hydrolysis of resulting glycine peptides by 6N HCI containing 0.4% β-mercaptoethanol (BME), at 110˚C for 24h gave glycine as the main product [20]. The confirmation and estimation of peptides were done by a modified biuret reaction based on the change in UV with λmax at about 263mm. This is attributed to the cupper-ion complex formation of the peptide in an alkaline medium.

Peptides were also estimated using Photochemical Colorimeter MK III at 570nm. Extending the duration of heating up to 100h, the quantity of glycyl-aspartic acid, gly^3, and gly^2 was relatively enhanced. The cyclic dimer DKP was also formed in better amounts. When the reaction system was heated up to 150h the quantity of all the resulting peptides enhanced and simultaneously the amount of aspartic acid decreased. Further, heating up to 200h resulted in the formation of identical reaction products. However, the quantity of glycyl-aspartic acid, DKP, and gly^2 slightly increased at the expense of lysine, aspartic acid, glycine, and gly^3. Finally, at 250h of heating, the best results were observed, and the quantity of all the peptides including DKP, lysine and α-alanine enhanced significantly. In contrast, a decrease in the amount of aspartic acid and glycine was illustrated in Figures 1 and 1a. The Control solution of a similar reaction mixture, analysed parallel to the reaction system failed to synthesise oligopeptides. Thus, the peptide bond formation (both cyclic and acyclic) and hydrolysis of amide bond were observed simultaneously by making and breaking processes (Scheme1) under drying/wetting cycles which might have been a common phenomenon in coastal regions of the primitive era.

Fig.1. Chromatogram showing the formation
of peptides from glycine-aspartic acid and water vapour
heated from 50 - 250 h (a-e) at 85 ± 5 ^{0}C under drying-wetting conditions

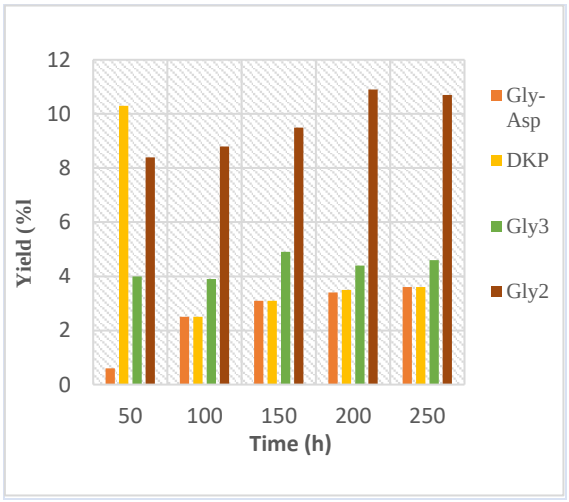
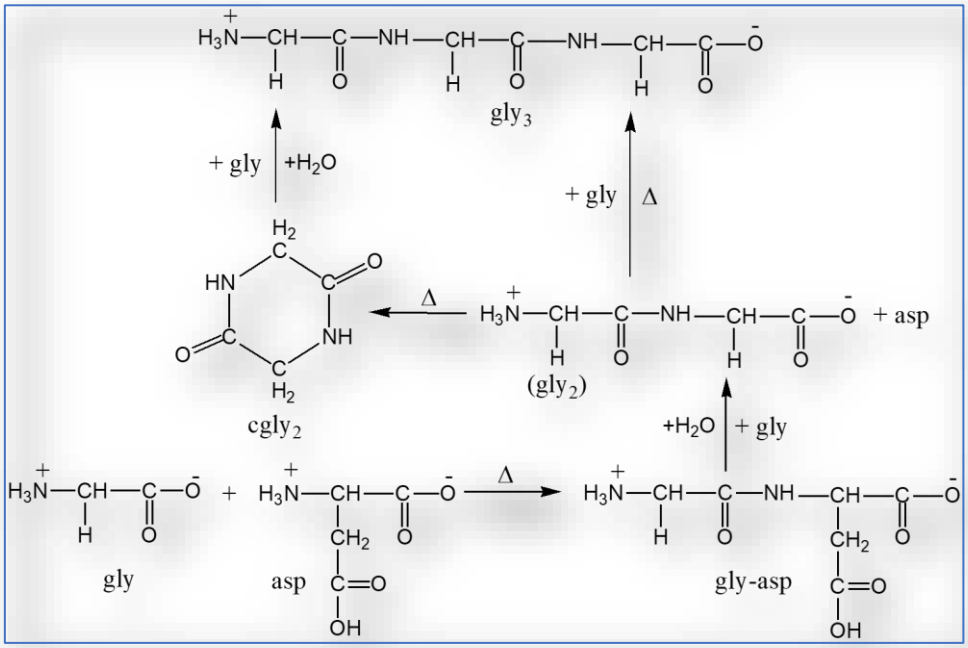


Fig. 1a Periodic heating analysis of resultant products up to 250 in percentage (%) yield under drying -wetting conditions



Scheme 1: Plausible pathways of glycine-aspartic acid reaction system published in our paper year 2009[7]

UV of the reaction concentrate of glycine aspartic acid, and water vapour heated up to 250h showed a band maximum at 220nm (not shown) indicating the presence of a mixture of peptides. The IR of identical solution showed absorption frequencies in a region of 3500-3450cm^{-1} due to NH_3 (asym. stret.), 1750-1690cm^{-1} due to C=O stret. in CONH2, 1650-1550cm^{-1} due to (asym. stret.) of COO^- and 1440-1360cm^{-1} due to COO^- (sym. stret.) and 1515 - 1490cm^{-1} due to C-N stret showed in Figure 2. The absorption frequency of C=O in the region of 1750-1690cm^{-1}, due to -CONH (amide group) indicates the formation of peptides in the above

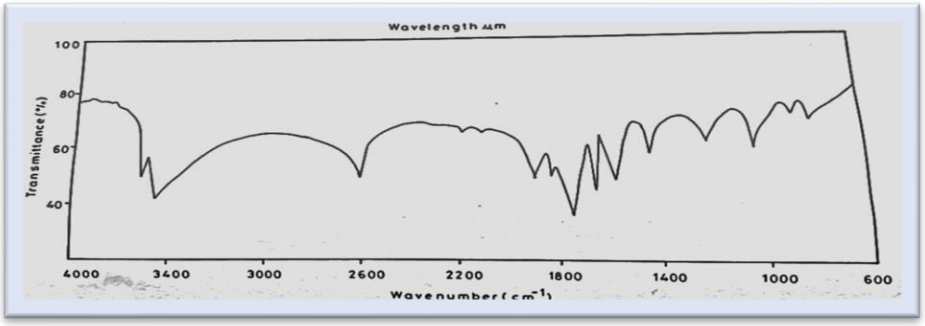


Fig.2. IR of glycine-aspartic acid -water vapour heated to 250 h. shows frequencies in regions of 3500-3450 cm^{-1}(N^+H_3 asym. stret.),1750-1690 cm^{-1} (C=O stret in $CONH_2$), 1650-1550 cm^{-1}(asym. stret. of COO^{-+}), 1440-1360cm^{-1} (COO^- sym. stret)

reaction system. The identity of the resulting peptides was further ascertained by HPLC, which showed twelve peaks. Out of which, peaks corresponding to the retention time of 2.204, 2.923, 3.590, 4.513, 5.129, and 7.130 min corresponded to gly, α-ala, gly^2, DKP, glycyl-aspartic acid, and gly^3 respectively (Fig.3). The peaks were perfectly matched with the retention time of standard samples run in same HPLC column under identical conditions.

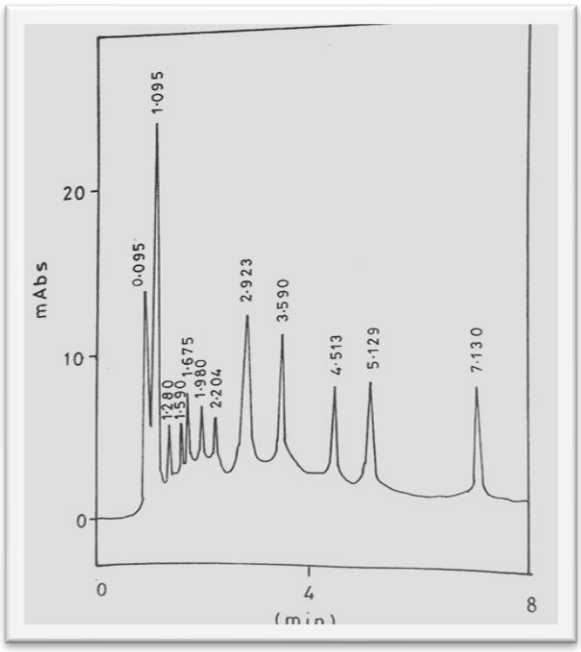


Fig.3. High-Performance Liquid Chromatography of glycine/aspartic acid concentrates and water heated for up to 250 h. The retention times 0.095, 1.095, 1.675, 2.923, 3.590, 4.513, 5.129 and 7.139 min corresponds to Asp, (gly)2, gly, (gly)2, (gly)3, gly-Asp, ala and lys, respectively.

Composition of Reaction system	**Product of Thermal reaction (Quantity in %)**						
	I	II	III	IV	V	VI	VII
Gly-Asp-H_2O(v)-SiO_2	19.9	20.4	19.9	19.0	+	22.4	0.41
Gly-Asp-H_2O(v)-Al_2O_3	17.1	18.6	17.9	18.0	0.13	21.4	0.43
1. Rf value (%) with	Physicochemical Characteristics						
(i)n-BAW,4:1:1v/v, at 18±2˚C	10	20.5	24	28	36	42	60
	.	2. Colour with Reagents					
(i) Ninhydrin	V	Y-V	Y-V	PV	V	V	V
(ii) Isatin	dB	LR	LR	P	BrV	BV	Bp
	3. Solubility						
(ii) Dil HCl	S	S	S	S	S	S	S

4. Amino acids /Peptides overlapped in co-chromatography	Asp	Gly^3	Gly^2	Gly	Thre	Ala	Leu
5. Products identified	Asp	Gly^3	Gly^2	Gly	Thre	Ala	Leu

Table 1 Physicochemical Properties of

Abbreviations used in the above table are as follows: V, Violet; R, Red; P, pink; Br, brown; d, dull; B, blue; p, purple; Y, yellow; S, soluble; Asp, aspartic acid; $Gly,^3$ triglycine $Gly,^2$ diglycine Gly, glycine; Thre, threonine, ala, alanine; Leu, leucine;

Fig.4. Chromatogram showing the formation of peptides from gly-asp and water vapour heated in the presence of silica and alumina

In this section effect of heat (85±5°C) on the reaction system of glycine-aspartic acid and water vapour (0.1M each, heated up to 250h) has been studied in the presence of silica as well as alumina separately (10mg each) as condensing agents. This study aimed to investigate the oligomerisation of glycine and aspartic acid on silica and alumina separately at temperatures below the boiling point of water under wetting/drying cycles of primitive Earth probably rich in these metal oxides. Chromatographic analysis of the reaction concentrates of a mixture of glycine, aspartic acid, and water vapour in silica resulted in seven ninhydrin-positive products. Out of which, products II and III were identified as gly^3 and gly^2 by comparison of their R_f values with standard gly^3 and gly^2 and various colour reactions with ninhydrin (yellow changing to violet) and isatin (light red). Products I and IV were detected as residual aspartic acid and glycine. No product corresponding to glycyl-aspartic acid and DKP were, however, detected on the paper gram. A few other amino acids such as threonine (V), α-alanine (VI), and leucine (VII) were also formed by thermochemical reactions of the reactant amino acids. When heating of a similar reaction system was carried out in the presence of alumina, no remarkable change in the number of products was noticed. The peptides gly^3 (II) and gly^2 (III) were formed in lesser amounts. The quantity of residual aspartic acid (1) and glycine (IV) was also decreased. However, the quantity of threonine (V), α-alanine (VI) and leucine (VII) were relatively enhanced residual aspartic acid (1) and glycine (IV) was also

decreased. However, the quantity of threonine (V), α-alanine (VI) and leucine (VII) were relatively enhanced. The physicochemical properties and quantitative yield (%) of the reaction products are recorded in Table 1 and depicted in Figures 4 and 5. Based on the number of peptides formed, the catalysing effect of silica was found relatively better than the peptides formed in the presence of alumina in the above reaction system heated up to 250h at 85±5°C. induced formation of peptides from a reaction mixture of glycine, valine, and water vapour in the silica/alumina under prebiotic wetting-drying cycles of primitive Earth

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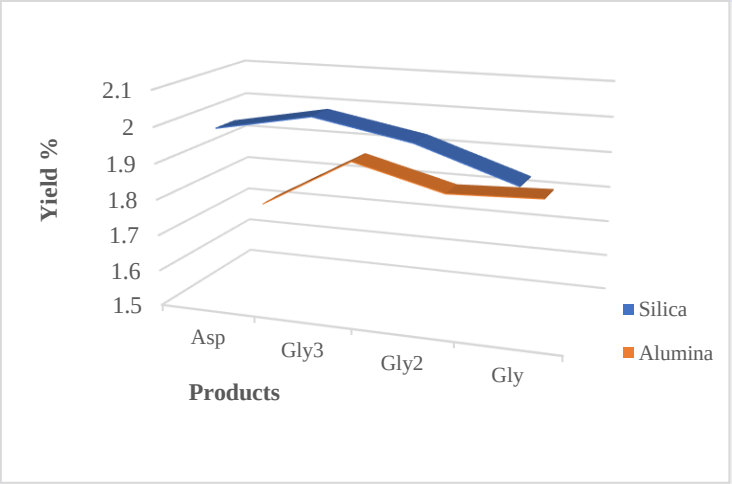


Fig. 5 Formation of peptides from gly/asp/water vapour in the presence of silica and alumina heating hour 250

Another reaction system of the synthesis of peptides has been carried from glycine (without any alkyl substituent at the α-carbon atom) and valine (with isopropyl group at the α-carbon atom) as the reactant amino acids. This study aimed to investigate the effect of branched-chain hydrophobic methyl groups on the formation of oligopeptides under primitive ocean-beach conditions. Analysis of the reaction concentrate of glycine-valine-water vapour heated for about 50 h resulted in seven ninhydrin-positive products (IV-X) on the paper chromatogram. 100h of heating produced one more product corresponding to product III. When the heating was continued for 150, a remarkable change in the number and the number of resulting products was observed. In all, ten thermal products were formed. Out of which, products I to IV and VI to VIII were identified as gly, gly, gly, gly and gly-val, val-gly, val² respectively employing their resolution factors and characteristic colours with ninhydrin (yellow changing to violet) and isatin (light red) and also by perfect coincidence between products and the standards. Products V and IX were characterized as residual glycine and valine. However, product X formed in trace amounts was identified as leucine. On extending the duration of heating to 200h, an identical range of products was formed, however, the number of products I and II remarkably decreased, while the quantity of the gly³ (III) relatively increased. On prolonging the duration of heating up to 250h, the number of products III and IV slightly increased, whereas the quantity of all other peptides including residual glycine and valine decreased shown in Fig.6,6a and Scheme II). The best products were observed when we heated the reaction system of glycine, valine, and water vapour to 150h under prebiotic wetting/drying conditions at 85±5°C. The blank reaction solutions produced none of the resulting oligopeptides.

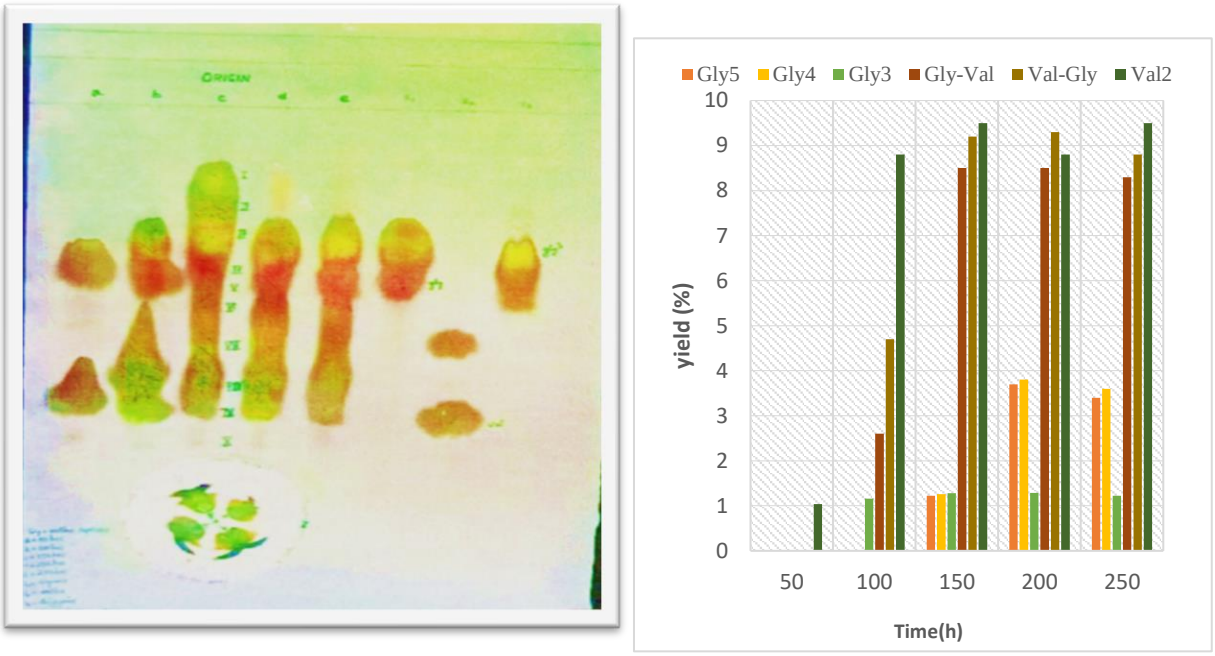


Fig. (6). Chromatogram showing the formation of peptides from glycine-valine and water vapour heated from 50-250 h (a-e) at 85 ± 5 ^{0}C in a terrestrial condition (I-gly$_5$, II-gly$_4$, III-gly$_3$, IV-gly$_2$, V-gly, VI-gly-val, VII-val-gly, VIII-val$_2$, IX-val and X-leu

UV spectra of the reaction concentrate of glycine, valine and water vapour heated up to 150h showed a band at 220nm (not shown) indicating the formation of peptides. The IR of identical solution showed absorption at 3350 cm-1 due to N-H stret (asym.), 3170 cm^{-1} due to N-H stret (sym.), at 1640 cm^{-1} due to absorption frequency of C-O stret. of -CONH group and at 1425 cm^{-1} due to C-N stret vibrations. The absorption frequency of C=O at 1640cm^{-1} due to -CONH (amide group) indicates the formation of peptides in the above reaction system (Fig. 7). Reaction concentrates of glycine, valine, and water vapour heated up to 150h were further analyzed by showed peaks at the retention time of 2.120, 3.532, 4.012, 4.265, 5.495, 5.910, and 7.510min corresponding to glycine, gly^2, valine, glycyl-valine, val^2, gly^4 and gly^5 respectively (Fig. 8). Results were perfectly matched with reference standards run parallel in the same HPLC column under identical conditions.

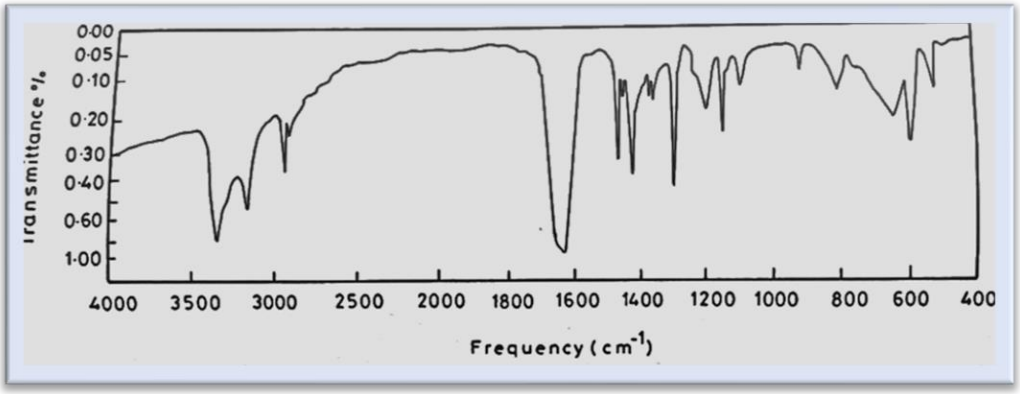
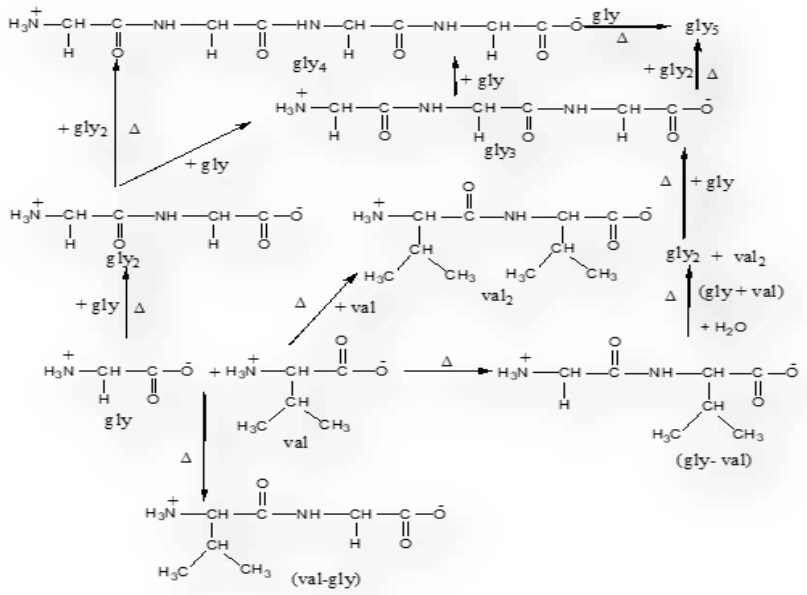


Fig.7. IR of glycine-valine and water vapour system heated up to 150 h show frequencies around 3350 cm^{-1} (N-H asym stret), 3170 cm^{-1} (N-H sym stret), 1640 cm^{-1} (C=O stret) and 1425 cm^{-1} (C-N stret).



Scheme 2: Plausible pathways of the glycine-valine-water vapour published in our paper in 2009[7].

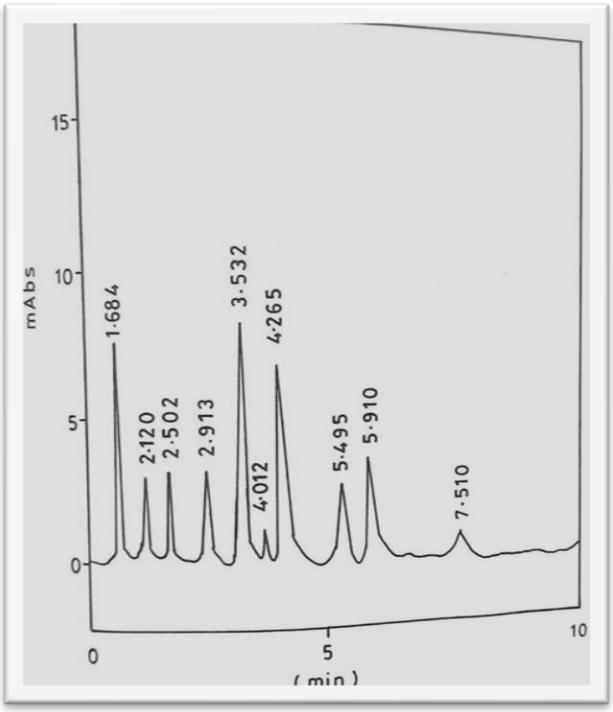


Fig. 8. High Performance of Liquid Chromatography of the reaction of glycine-valine-water vapour heated up to 150 h The retention time 2.120, 3.532, 4.012, 4.265, 5.495, 5.910, and 7.510min corresponding to glycine, gly^2, valine, glycyl-valine, val^2, gly^4 and gly^5 respectively.

The effect of heat on the reaction mixture comprised of glycine, valine (0.1M of each), and water vapour separately in the presence of silica and alumina has a bed of under simulated primitive Earth conditions to understand the role of metal oxides available in abundance near sea-beaches on the synthesis of peptides and other related products. The reaction solution was heated up to 150h at 85±5°C and the concentrates were analysed for the formation of peptides. The heated reaction mixture of glycine, valine, and water

vapour in the presence of silica/alumina as a condensing agent revealed the formation of oligopeptides along with some other amino acids. When heating was carried out in the presence of silica, the formation of nine products was observed on the paper gram. Products III, IV, and VII, formed in appreciable amounts were identified as gly^4, $gly^{2,}$ and valinyl -glycine respectively. In contrast, products, I, II, VI, and IX detected in moderate amounts were characterized as gly^4, DKP, glycyl-valine, and leucine. Products V and VIII were found as residual glycine and valine. The presence of alumina during heating under identical conditions caused an increase in the concentration of DKP (II), gly^4 (III), gly^2 (IV), glycyl-valine (VI) and valinyl-glycine (VII) shown in Fig.9 and Table2. Eluting the ninhydrin and isatin-positive spots in double distilled water and then measuring the optical density in MK- III Photochemical Colorimeter at 570nm wavelength estimated the colour intensities of the spots. Based on the nature of the resulting products and their quantity, the catalysing effect of silica and alumina on the above reaction system.

TABLE-2 PERCENT (%) YIELD OF PEPTIDES FORMED FROM THE REACTION SYSTEM OF GLY/VAL/WATER VAPOUR IN PRESENCE OF SILICA AND ALUMINA

Composition of Reaction system	**Product of Thermal reaction (Quantity in %)**								
	I	II	III	IV	V	VI	VII	VIII	IX
Gly-Val-H_2O(v)-SiO_2	0.41	0.81	10.9	12.5	12.4	0.95	12.3	11.6	0.09
Gly-Val-H_2O(v)-Al_2O_3	0.06	8.80	12.2	12.6	12.3	0.93	12.4	12.0	0.07
1. Rf value (%) with	Physicochemical Characteristics								
(i)n-BAW,4:1:1v/v, at 18=20c	4.9	8	12	17	26.2	32	38	45	52
(ii)n- n-BAW,4:1:5v/v upper layer	8	12.5	15.6	22	28	34	40	47	56
	2. Colour with Reagents								
(i) Ninhydrin	Y-V	Y-R	Y-V	Y-V	V	V	Y-V	V	V
(ii) Isatin	LR	R	LR	LR	P	R	R	Brp	RV
	3. Solubility								
(i)Ether	Ins	Ins	Ins	Ins	Ins	Ins	Ins	Ins	-
(ii)Dil HCl	-	S	S	S	S	S	S	S	-
4. Amino acids /Peptides overlapped in co-chromatography	-	-	Gly_3	Gly^2	Gly	-	-	Val	-
5. Products identified	Gly^4	DKP	Gly_3	Gly^2	Gly	Gly-Val	Val-Gly	Val	Leu

Abbreviations are similar to those above in TABLE 1

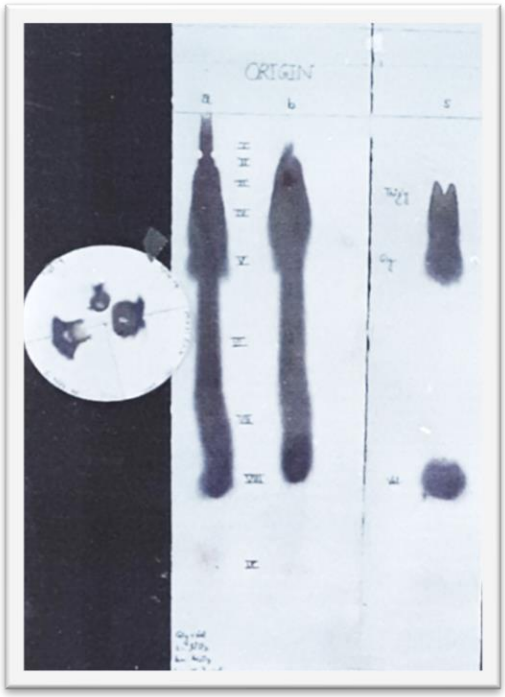


Fig 9 Chromatogram showing the formation of peptides from glycine-valine and water vapour heated upto150h under drying-wetting conditions in the presence of silica and alumina

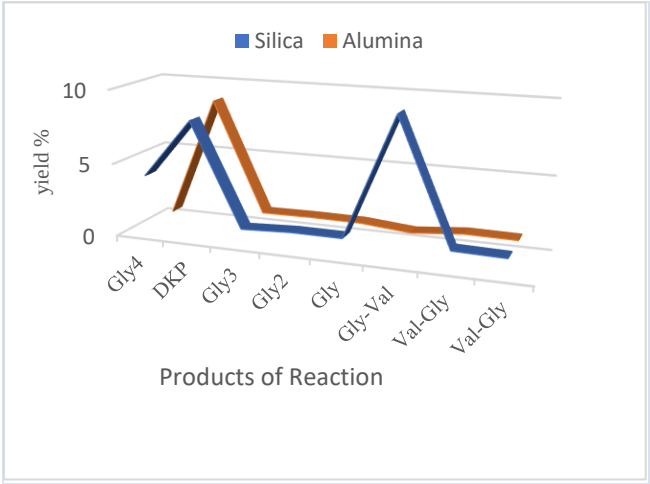


Fig. 9a Formation of peptides from gly/asp/water vapour in the presence of silica and alumina heating hour 150

Conclusion

When glycine is heated singly under anhydrous conditions, it pyrolyzes into a blackish-brown solid without forming peptides. However, in an aqueous solution, glycine forms peptides such as gly2 and cyclic gly2 under drying and wetting conditions. When glycine is heated with aspartic acid, the amount of gly2 decreases, while the amount of gly3 and gly-asp increases. This suggests that aspartic acid initiates the formation of glycine peptides up to the trimer level. Aspartic acid activates the carboxylic end of glycine through anhydride formation, allowing it to react with free glycine molecules to form gly2 and cyclic gly2. The hetero-peptide gly-asp is formed through condensation of the carboxyl end of glycine with the amino

end of aspartic acid. The presence of silica accelerates the formation of gly3 at the expense of gly2 but inhibits the formation of cyclic gly2. The difference in peptide formation between the gly/asp and gly/val systems can be attributed to the unique properties of each amino acid. Aspartic acid's active site in the gly/asp system reacts with the silanol group, leaving glycine molecules to form peptides through intermolecular condensation. In contrast, the gly/val system shows that valine's neutral and hydrophobic nature accelerates the formation of glycine peptides up to the pentamer level (gly^5). This is due to the branched-chain isopropyl group in valine, which facilitates interactions with glycine through dipole-dipole interactions. As a result, more gly-val peptides are formed than val-gly peptides. The isopropyl group in valine also affects its electronic properties, increasing the electron density on the carboxylate end and decreasing the positive charge on the ammonium end. This enhances the nucleophilic activity of the NH_4^+ and reduces the electrophilic activity of the carboxylate. Overall, the study suggests that combining valine with glycine favours the formation of glycine peptides up to the pentamer level, whereas combining aspartic acid with glycine does not [7]. The product yield in the reaction system involving glycine, valine, and water vapour remains unchanged, but the diversity of homo-peptides and hetero-peptides increases. Furthermore, we introduce condensing agents like silica and alumina in this reaction system to enhance peptide formation. Notably, alumina is significantly better than silica in facilitating the formation of peptides up to the tetramer level and, likewise the generation of hetero-peptides.

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