Heat Induced Formation Of Peptides From Reaction Mixture Of Glycine - Glutamic Acid And Glycine-Leucine In Presence And Absence Of Montmorillonite Clay With Or Without Metal Ions Under Wetting Drying Cycles Of Primitive Earth

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Abstract:- The effect of heat on the reaction system of glycine - glutamic acid and glycine- leucine at 90 ± 5^0C has been investigated in aqueous environment in the presence of montmorillonite clay with or without divalent cations (Ca^{2+}, Cu^{2+} and Mg^{2+}) under prebiotic wetting-drying cycles of primitive earth. The resulting products were analyzed by paper chromatography, UV as well as by High Performance Liquid Chromatography. Formation of peptides seems to depend on the nature of the reactant amino acids, duration of heating as well as on montmorillonite clay incorporated with divalent cations. In glycine/glutamic acid, oligomerization of glycine was limited upto tetramer level (Gly_4) along with the formation of glycyl-glutamic acid, whereas reaction system of glycine/leucine gave peptides up to tetramer level (Gly_4) and showed the formation of Leucyl-Glycine (Leu- Gly). Thus the formation of peptides from the above reaction system reveal that incorporation of metal ions on clay (M) surface enhance the catalytic activity by ion-dipole interaction of cations with dipolar amino acid Zwitter-ions.

Keywords:- Prebiotic,diagenesis,biopesis,oligimerisation,spectrophotometer,micromolecules,divalent.

Introduction:

Life is considered to be a logical consequence of the physico-chemical interactions that have been going on ever since the universe came into being. All present day life is all its variations is based on nucleic acid, proteins, carbohydrates, fats and on some minor compounds such as phosphoric esters. The postulated stages of chemical evolution leading to the first speck of life include the formation of organic molecular and gaseous constituents and their accumulation in the primordial seas. Although much knowledge has been gained in recent years in the study of the abiotic origin of life, many questions remain. Most of these questions centre on the mechanism whereby small biological molecular, such as amino acid and nucleic acid bases, become organized into a system of polymers which evolved into catalysts, templates and self- replicating system. Because of their wide distribution in geological time and space and their strong affinity for organic compounds, clay and clay minerals are most likely candidates among solid materials to have contributed to chemical reactions producing the polymeric substances from which life emerged. The importance of clay minerals in chemical evolution was suggested by Bernal in 1951.

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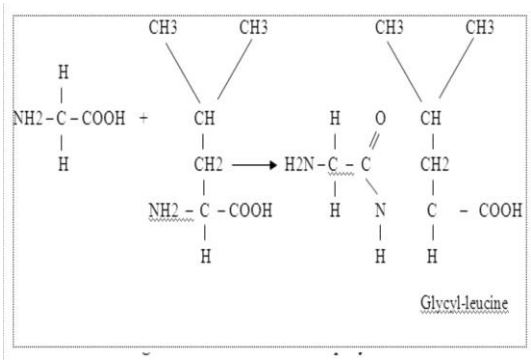
He proposed that clays near the hydrosphere-lithosphere interface might have adsorbed organic micromolecules, thereby providing high local concentration of reactants needed to form certain biologically important micromolecules. As soon as liquid water appeared on the surface of primitive earth, clay minerals accumulated on the surface and suspended into deep sea. Bernal has emphasized that clays, minerals and silicates have played a vital role in chemical evolution and suggested that clay near the hydrosphere-lithosphere interface might have adsorbed micro bio monomers on and between their silicates layers, thereby protecting them from destructive radiations from the sun. Rao et. al. and Ponnamperuma et.al. have reviewed the possible role of clay minerals (montmorillonite, kaonilite and bentonite) in the process leading to the origin of life and strengthened Bernal's hypothesis. Clay minerals are formed during diagenesis of sediments and also by igneous activity as an alteration product of silicate minerals. In montmorillonite clay $[(Al)\ (Mg)_2\ OH_2]^{-1}\ [Si_4O_{16}]\ [Na\ (Ca)_n\ H_2O]^{+1}$, the two tetrahedral silica sheets are sandwitched on alumina sheet occupying two third of the octahedral sites leaving one third of the sites vacant. Grim proposed that during weathering the divalent cations such as Mg^{2+} may replace Al^{3+} in the octahedral sheet may be replaced by trivalent cations such as Al^{3+}. Further the oligomeuzation of amino acids is considered to be a plausible step in the biopesis of complex biomolecules such as peptides and subsequent formation of proteins. Earlier Fox and Harada and others have shown that amino acid polymerise under the influence of heat at elevated temperature. Chang, Flores and Ponnamperuma have studied that effect of ultraviolet light on aqueous solution of simple amino acids i.e. glycine and leucine in the presence of condensing agents e.g. cyanamide or a tetramer of hydrogen cyanide and reported the formation of diglycine, triglycine, glycyl-leucine and leucyl-glycine.



Other investigators have demonstrated polymerization of amino acids at elevated temperatures in aqueous solution at temperatures from 130^0 to 160^0C. Nagayama et. al. and Baswik et. al. have shown that linear glycine peptides can be formed from diketopiperazine in aqueous solution at 90^0C. Rode and Schwendinger carried out the polymerization of glycine in sodium chloride solution containing Cu^{2+} at 70^0 to 100^0C. Yanagawa and Kojima, Yaragawa et. al. and Yanagawa and Kobayashi were successful in synthesizing peptide like polymers at temperatures> 250^0C. Bernal suggested that formation of biopolymers could have been catalyzed by solid compounds, especially minerals common in nature. The role of such solid minerals as catalysts was claimed as concentrating monomers on their surface, catalyzing the formation of bioplymers and protecting them against hydrolysis as suggested by Lawless and Levi, Theng reported that amino acids are not well adsorbed on clay surfaces as compared to short oligopeptides. However, Bujdak et. al. have suggested that the role of clay minerals was mainly in the chain elongation of oligopeptides rather than the formation of peptides from amino acids. Earlier a number of experiments have been carried out for the synthesis of peptides from amino acids catalysed by clay minerals such as montmorillonite, kaolinite, bentonite and hectorite etc. Paecht-Horowitz reported that montmorillonite catalyse peptide bond formation from aqueous solution of an activated amino acid adenylate. However, Warden, et. al. impose doubt on formation of such activated amino acids under prebiotic conditions. Although the formation of peptides by heating amino acids on clays above 100^0C was observed by Friepiat, et. al. Raki, Yanagawa and Kojima, yet the best yield of glycine oligopeptides was reported at 94^0C by Bujdak et. al., White and Frickson et. al. Similar experiments were carried out by Lawless and Levi in presence of cation exchanged (Cu, Zn, Ni and Na) clays to study the effect of metal ions. Experiments concerning adsorption of amino acids and their peptides on solid surfaces under prebiotic conditions have been carried out. (Kalra et. al. 2000, 2003; Meng. et. al. 2004; Zaia 2004; Whitehouse et. al. 2005; Lambert 2008; de Pavia et. al. 2008). Synthesis of biomonomers, their subsequent adsorption on solid surfaces under prebiotic conditions provide insights in our present day understanding of the basic theory of molecular evolution leading to the origin of life. Therefore, in our present investigation two different reaction system consisting of glycine in combination with glutamic acid and glycine with leucine as reactants has been studied in aqueous medium for the possible synthesis of peptides in the presence and absence of montmorillonite clay with or without divalent cations (Ca^{2+}, Mg^{2+} and Cu^{2+}) under wetting-drying conditions presumed to be available near the hydrosphere-lithosphere boundary of the primitive sea.

Materials and Methods: -

Montmorillonite clay (E. Merck) was purified by sedimentation in water and the purity of a < 2μm sample was tested by x-ray diffraction measurement, Homo ionic clays (Ca^{2+}, Mg^{2+}, Ca^{2+}) were prepared by the saturation method (Theng 1974) with 50 ml of 1 M concentration of each metal chloride. The cation exchanged clays thus prepared were repeatedly washed with double- distilled water and centrifused for 10 minutes in Remi 3500 rpm until freed of chloride ion and were used in all cases studied. All the chemicals including amino acid and reference oligopeptides were from Sigma Chemical Company. Sterilized aqueous solution of a mixture of glycine and glutamic acid as well as glycine and leucine (5 mM, 0.1 M each) were taken separately in Borosil glass reaction vessels (100 ml) fitted with air condensers and kept on heating plates at a temperature of 95 ± 5^0C. Evaporation cycles were performed in order to simulate ocean- beach conditions in the presence and absence of montmorillonite clay with or without divalent cations i.e. Ca^{2+}, Mg^{2+} (0.01 g each). The reaction mixture was heated leading to complete evaporation within 8 to 10 hrs/ day in each cycle and the next evaporation cycle was performed by adding 10 ml distilled water. Control experiments were set by keeping experimental samples of each reaction (10 ml) kept in Borosil glass containers (25 ml) wrapped heavily in black cloth and kept separately. Wetting and drying cycles were continued for varying periods up to 250 hrs and the reaction concentrates as well as the control solution were analyzed periodically (50, 100, 150, 200 and 250 hrs) by paper chromatography (Hais and Macke 1963) and high performance liquid chromatography (HPLC) (Hancock and Harding 1982) methods. The reaction concentrates heated up to varying periods in presence of montmorillonite clay with or without divalent cations (Ca^{2+}, Mg^{2+}, Cu^{2+}) were shaken in aqueous $CaCl_2$ solution to release the resulting peptides prior to analysis. The analysis of samples of different reaction concentrates heated for varying periods up to 250 hrs were carried out on Whatman No. 1 filter paper using n- butanol- acetic acid- water (4:1:1 v/v) as the solvent system and ninhydrin as the color producing reagent. Shimadzu SPD-10 A, UV- vis- detector monitored at 210 nm with C_{18} column was used to analyze the resulting peptides and other amino acids in all cases investigated. The mobile phase was 25% CH_3CN : 75% Na_2HPO_4, pH adjusted to 2.5 with H_3PO_4 at 20-25^0C and the flow rate monitored at 1.0- 1.2 ml/min. The identity of the resulting products was ascertained by Rf values, color with ninhydrin, UV using Jasco V- 550 spectrophotometer, IR using Perkin Elmer FT-IR spectrophotometer and HPLC retention time. The identity of the resulting products were compared with the spectra of the authentic reference standards. The optical density of the colored spots was measured by using MK III calorimeter monitored at 570 nm. The reaction yields were determined as a percentage of the reactants converted to the reaction products. The results have been recorded in Tables 01 and o2 and recorded in Fig A and B.

Results: -

Two parallel reactions i.e. glycine-glutamic acid/ glycine-leucine in the presence and absence of montmorillonite clay with or without divalent cations (Ca^{2+}, Mg^{2+}, Cu^{2+}) under wetting and drying conditions were investigated for the possible formation of peptides. Chromatography of the reaction concentrate of glycine- glutamic acid with or without catalyst heated upto 150 hrs showed the formation of tetraglycine, triglycine, diglycine and glycyl- glutamic acid (Fig.A). Time lapse studies of the reaction concentrate heated for a period of 50 hrs showed the formation of (Gly_3) and (Gly_2) in significant quantity. A remarkable increase in the quantity of Gly- Glu (Gly_3) and (Gly_2) was observed when the reaction concentrate was heated for a period of 100 hrs. On prolonging the duration of heating upto 150 hrs gradual increase in the formation of the products was observed. The results are recorded in table 01. The results of the above reaction mixture were further confirmed by the hydrolysis of the resulting peptides by 6 N, HCl which gave glycine as the main product. The confirmation and estimation of peptides was done by modified biuret reaction based on the change in the UV spectrum with a maximum at about 263 nm, which is attributed to the copper ion complex of the peptide in alkaline medium. Colorimetric estimation of peptides was also carried out at about 570 nm. The results were further confirmed by High Performance Liquid Chromatography on SHIMADZU SPD 10 A apparatus which showed four major peaks. Out of these peaks corresponding to retention times of 1.728 min and 3.530 min matched with authentic diglycine and triglycine respectively, run under identical conditions. (FigC) The reaction system of glycine- glutamic acid heated under prebiotic wetting-drying condition of primitive earth in the presence of montmorillonite clay without divalent cations heated for a period of 150 hrs showed the formation of Gly-Glu, (Gly_4), (Gly_3) and (Gly_2) along with residual reactants. When the effect of heat was further carried out in the presence of Mg^{2+} exchanged montmorillonite clay, the quantity of triglycine (Gly_3), diglycine (Gly_2) and glycyl-glutamic acid (Gly-Glu) was relatively increased. However, the product corresponding to tetraglycine (Gly_4) was not detected on the papergram. The same reaction system in the presence of Ca^{2+} exchanged montmorillonite clay showed the appearance of (Gly_3), (Gly_2) and Gly-Ghu in good amount. On the other hand the same reaction system in the presence of Cu^{2+} incorporated montmorillonite clay showed the formation of an identical range of products in relatively better amount. Results recorded in Table 01 and illustrated in (Fig. A) clearly indicate that the reaction system of glycine- glutamic acid incorporated with Ca^{2+} clay resulted in the formation of peptides in relatively higher amount. Another reaction system of glycine and leucine was carried out to know the effect of branched chain hydrophobic methyl groups on the promotion of oligopeptides under primitive ocean-beach conditions. The results are recorded in Table 02 and illustrated in Fig. B. The reaction system heated upto 200 hrs under prebiotic wetting-drying condition on chromatographic analysis showed the formation of linear peptides of glycine upto tetramer level along with the formation of Leu-Gly. When the reaction system of glycine and leucine heated upto 50 hrs on chromatography gave Leu-Gly in good amount while other products viz. (Gly_4), (Gly_3) and (Gly_2) in lesser amount. The amount of (Gly_4) and (Gly_3) was enhanced on heating the reaction system upto 100 hrs along with the formation of (Gly_2) and Leu-Gly. At 150 hrs of heating, formation of homo and hetero peptides (Gly_2) to (Gly_4) was detected along with the formation of Leu-Gly in increased amount. A slight decrease in the amount of almost all the peptides was noticed on analysis of the reaction concentrate at 200 hrs of heating. HPLC analysis of the reaction concentrate of glycine-leucine heated upto 150 hrs showed nine peaks. The peaks corresponding to retention time of diglycine (1.813 min), triglycine (3.52 min), tetraglycine (4.512 min) and leucyl-glycine (5.291 min) were exactly matched with authentic diglycine, triglycine, tetraglycine and leucyl-glycine respectively, run under identical conditions. (Fig D). The UV absorption spectra of the reaction mixture showed a band lying at 218 nm indicating the formation of a mixture of peptides(Fig E). The formation of peptides were further confirmed on the basis of i.r. spectrum of the heated concentrate of the reaction mixture of glycine, glutamic acid/leucine and water vapour under wetting-drying cycles of primitive earth near hydrosphere-lithosphere boundary in presence and absence of montmorillonite clay with or without divalent cations. In almost all the reactions studied, the free N-H group showed absorption in the region 3050-355 cm^{-1} and 1600-1640 cm^{-1}. The absorption band in the region 1640-1690 cm^{-1}, 1250 cm^{-1}, 1680-1725 cm^{-1} and 2500 cm^{-1} also indicated the presence of C=O, -CONH and –COOH group (Fig F). In the presence of montmorillonite clay with or without divalent cations (Ca^{2+}, Cu^{2+} and Mg^{2+}) slight changes were observed in the formation of the resulting peptides. (Table 02). (Gly_3), (Gly_2) as well as Leu-Gly were formed in appreciable amount while the other products were formed in pror amount. Presence of calcium (Ca^{2+}) exchanged montmorillonite clay during the course of heating in the above reaction system accelerated the formation of diglycine, glycine, leucyl-glycine and leucine and retarded the formation of triglycine. Experiment carried out in the presence of copper (Cu^{2+}) incorporated clay showed the formation of triglycine and the quantity of all the resulting peptides enhanced. The presence of Mg^{2+} exchanged montmorillonite clay revealed the formation of diglycine and leucyl-glycine in relatively better amount whereas formation of triglycine was not detected on the papergram (Fig B).

Discussion: -

From the results discussed above, it is revealed that glycine (the simple amino acid most easily formed under many of the prebiotic processes might have easily combined with other amino acids to form oligopeptides under evaporation cycles near hydrosphere- lithosphere boundaries of primitive sea. A comparative analysis has shown that he oligomerisation of glycine itself into tetraglycine, triglycine and diglycine in appreciable amount occurs in presence of neutral amino acid (leucine) rather than acidic amino acid (glutamic acid). Formation of glycyl-glutamic acid does not occur without the activating catalysts such as montmorillonite clay while leucyl- glycine is formed even in absence of catalyst under identical conditions of heating. It was also observed that the incorporation of metal ions on clay (M) surface enhance the catalytic activity by ion-dipole interaction of cations with dipolar amino acid zwitter-ion.

TABLE 01

Percentage yield of peptides formed from reaction system of glycine/glutanic acid and water

Duration of heating	Gly-Glu	(Gly_2)	(Gly_3)	(Gly_4)
50 hrs	-	**0.15**	**0.11**	-
100 hrs	-	**0.19**	**0.16**	-
150 hrs	-	**0.23**	**0.14**	-
200 hrs	-	**0.20**	-	-
250 hrs	-	**0.19**	-	-
M (150 hrs)	**0.04**	**0.45**	**0.42**	**0.23**
Ca^{2+} + -M (150 hrs)	**0.18**	**0.60**	**0.64**	-
Cu^{2+} + -M (150 hrs)	**0.25**	**0.64**	**0.72**	**0.18**
Mg^{2+} + -M (150 hrs)	**0.08**	**0.58**	**0.50**	-

TABLE 02

Percentage yield of peptides formed from reaction system of glycine/leucine/ water

Duration of heating	Gly-Glu	(Gly_2)	(Gly_3)	(Gly_4)
50 hrs	**0.17**	**0.06**	**0.07**	**0.05**
100 hrs	**0.16**	**0.08**	**0.09**	**0.08**
150 hrs	**0.40**	**0.18**	**0.23**	**0.11**
200 hrs	**0.16**	**0.12**	**0.08**	**0.08**
M (150 hrs)	**0.08**	-	**0.05**	**0.04**
Cu^{2+} + -M (150 hrs)	**0.08**	-	**T**	**0.06**
Ca^{2+} + -M (150 hrs)	**0.09**	-	**0.03**	**0.10**
Mg^{2+} + -M (150 hrs)	**0.10**	-	**T**	**0.12**

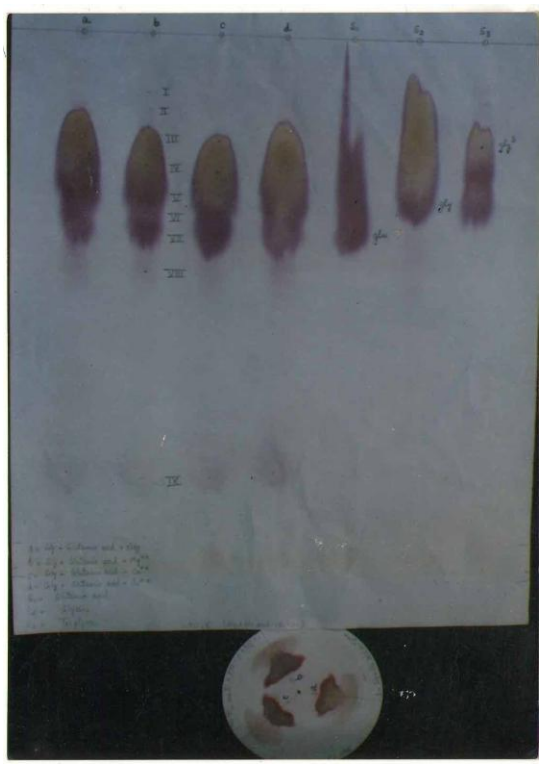


Fig A: Chromatogram showing the formation of peptides from reaction system of Glycine –Glutamic acid heated upto 150 hrs.

Fig B:Chromatogram showing the formation of peptides from reaction system of Glycine and Leucine heated upto 150 hrs.

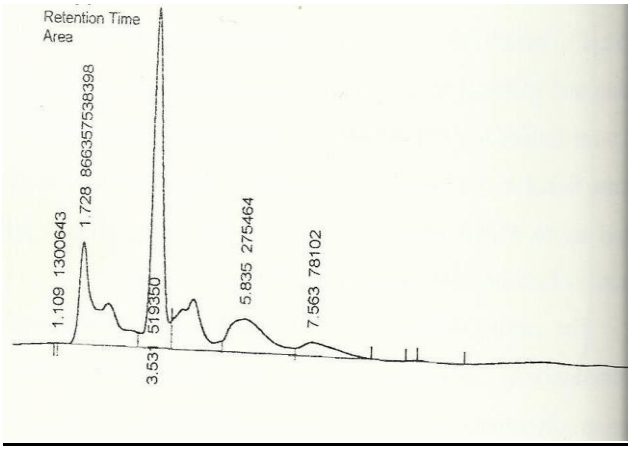


Fig (C) HPLC of reaction concentrate of gylcine-glutamic acid heated upto 150 hrs.

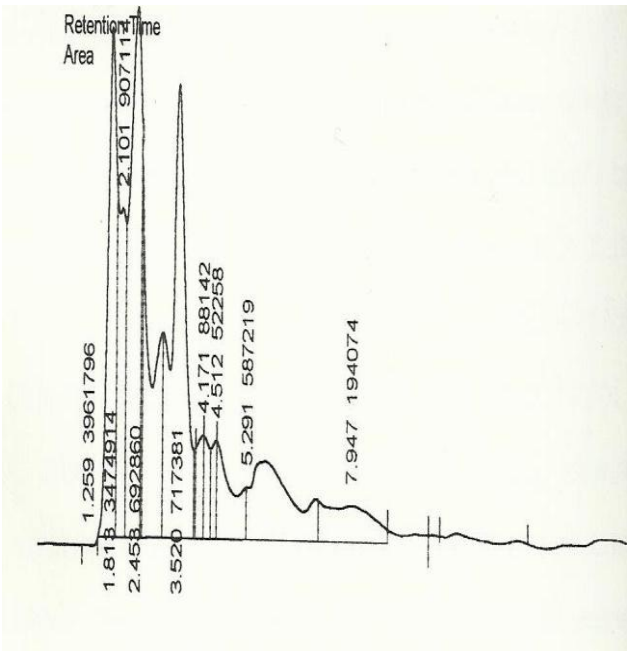
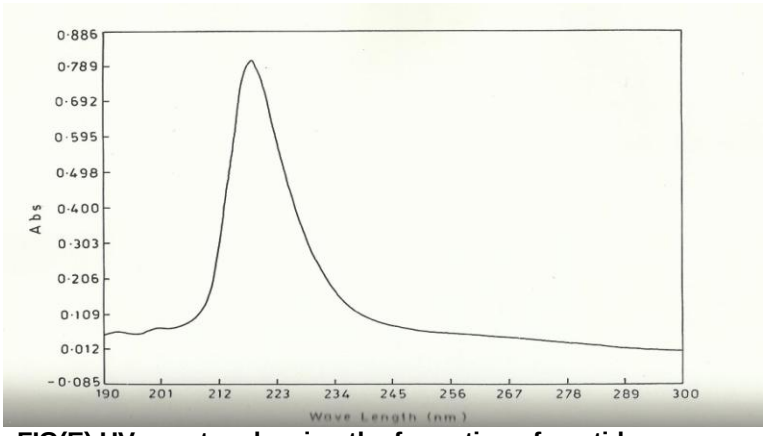


Fig (D) HPLC of reaction concentrate of glycine – leucine heated upto 150 hrs.



FIG(E) UV spectra showing the formation of peptides.

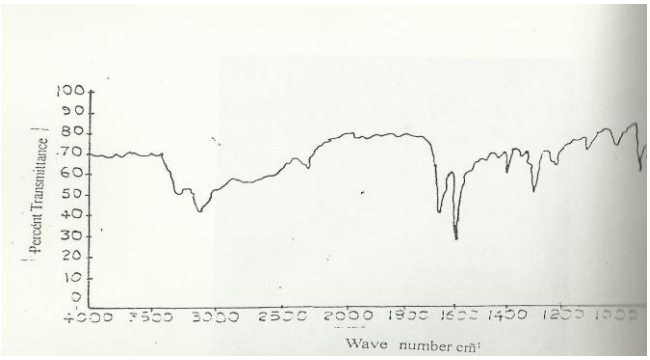


FIG (F) IR spectra showing the formation of peptides.

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