A POSSIBLE PREBIOTIC PEPTIDE FORMATION FROM GLYCINAMIDE AND RELATED COMPOUNDS

Hiroshi YANAGAWA, Masato NISHIZAWA and
Kiyotsugu KOJIMA

Mitsubishi-Kasei Institute of Life Sciences,
11 Minamiooya, Machida-shi, Tokyo 194, Japan

Abstract: We examined an experimental approach to the genesis of protocells in the primeval sea. Glycine polymers with an average chain length of 12 were formed from glycinamide in fluctuating systems (pH 7.2, 80°C, 20 cycles). The resulting glycine polymers gave aggregated leaflet-like structures. A solution of the glycine polymers provided stacked disc-shaped structures in the presence of LiBr and gave sheet structures in the presence of dichloroacetic acid. The shapes of these organized structures were correlated with their molecular structures.

A phase boundary or envelope which isolates a portion of the environment is essential for the genesis of life. In aqueous systems a phase boundary must be hydrophobic. One can make envelopes of self-assembled lipids by casting lipids onto a disturbed water surface. Oparin's coacervate (1) and Fox's proteinoid microsphere (2) demonstrated that envelopes self-assemble in aqueous systems from partly hydrophobic materials.

Protocells would have been formed from polymeric materials including lipids, proteins and carbohydrates in the primeval sea. Egami has recently found that a close correlation exists between the concentrations of different chemical elements in contemporary sea water and their biological behavior; he postulated that transition elements that are relatively abundant in sea water such as molybdenum, iron and zinc must have played important roles in the course of chemical evolution (3). Based upon this idea, we have been trying an experimental approach to the chemical evolution in the primeval sea. In the course of the

Origins of Life 14 (1984) 267–272. 0302-1688/84/0141-0267$00.90

research we have found that organized particles, "marigranules", were formed from glycine and acidic, basic and aromatic amino acids in a modified sea medium (4). The marigranules had the ability to synthesize structure protein-like macromolecules from amino acids.

Our additional aim has been to build up a good model system for the prebiotic synthesis of various proteins including structural proteins and enzyme proteins in the primeval sea. Our specific goal has been to use some fundamental amino acids such as glycine, alanine, aspartic acid and valine to produce phase boundary layers or envelopes and enzyme proteins in aqueous systems. Glycine, alanine, aspartic acid and valine were probably formed in the early stages of chemical evolution (5-8). Glycine is the most fundamental amino acid among these amino acids and it is most abundantly formed in the prebiotic synthesis of amino acids. A high concentration of glycine has been found in many biologically important structural proteins such as collagen (9), elastin (10), fibroin (11) and ligament protein (12). Furthermore, glycine was quite recently found in the protein fraction of black smoker bacteria which grow at 250°C, 265 atm (13). We have recently found that several glycine oligomers were formed up to a hexamer in a neutral aqueous solution by using Gly-NH_2 (14), an intermediate in the formation of glycine by the Strecker synthesis (15). In this paper, we describe the formation and morphology of glycine polymers which were produced from Gly-NH_2 in fluctuating systems.

Gly-NH_2·HCl (Sigma) was used without further purification. Glycine polymers were synthesized from Gly-NH_2 in fluctuating systems. The following buffer solutions (0.1 M, pH 7.2) were used: Na_2HPO_4-NaH_2PO_4, $KHCO_3$-NaH_2PO_4 and imidazole-HCl. A reaction mixture (10 ml) containing 0.1 M Gly-NH_2·HCl and one of the above buffers was placed in a beaker (100 ml, ϕ60 mm × 70 mm) and heated at 80°C. The solution was completely evaporated to dryness by heating for an hour, and it was then heated on a solid phase at 80°C. After heating for an hour, the same reaction mixture (10 ml) was added to the resulting powder, adjusted to pH 7.2 with hydrochloric acid, and heated at 80°C for an hour. This series of operations was repeated 20 times. The resulting powder was extracted with water and fractionated to give a water-soluble part and a water-insoluble part. The amino acid analysis of the water-soluble part provided glycine oligomers up to octamer. A quantitative analysis of glycine oligomers up to pentamer was performed by amino acid analysis; however, a sufficient amount of the hexamer or above gave a peak too small to allow for an exact calculation. The fluctuation reaction in an imidazole-HCl buffer system gave the best yield of glycine oligomers of up to octamer. The water-insoluble part was completely dissolved in 10 M LiBr solution and applied to gel

filtration (Biogel P-2) to estimate the molecular weight of glycine polymers. They were eluted in a single peak with an average chain length of 12. The average chain length was also measured by ^{13}C-NMR spectroscopy and it was calculated to be 12. Thus, glycine polymers with an average chain length of 12 were obtained from glycinamide in a fluctuating system ($KHCO_3$-NaH_2PO_4 buffer, pH 7.2, 80°C, 20 cycles).

The formation of organized structures from the resulting glycine polymers was studied by scanning electron microscopy. Glycine polymers with an average chain length of 12 (1 mg) were suspended in 0.1 ml of water, applied to a cover glass, dried at room temperature, and observed with a scanning electron microscope (JEOL 100CX). The polymers provided aggregated leaflet-like structures (Fig. 1). On the other hand, the glycine polymers (1.7 mg) were dissolved in 0.03 ml of 10 M LiBr solution and then slowly diluted with 0.4 ml of distilled water. A significantly turbid solution was thus obtained. This turbid solution (0.32 ml) was mixed with 0.08 ml of 25% glutaraldehyde solution and allowed to stand at room temperature. After standing for an hour, the phase-separated organized structures were collected by centrifugation at 3000 rpm for 20 min. The precipitate was washed with distilled water 3 times and dried on a cover glass at room temperature overnight. A scanning electron micrograph of this sample is shown in Fig. 2. The glycine polymers produced disc-shaped structures which were stacked in aggregates. The disc was 5 μm in diameter. This picture is distinguishable from that of the marigranules formed from amino acids in a modified sea medium (4). When fixation with glutaraldehyde was omitted, an isolated disc-structure was observed instead of the stacked disc-shaped structures. When the glycine polymers (5 mg) were dissolved in 3 ml of dichloroacetic acid and then added dropwise to 5 ml of dioxane with stirring, organized structures were phase-separated. They were rather different from those observed in the 10 M LiBr solution: sheet structures were formed (Fig. 3). These differences may be correlated with the molecular structures of glycine polymers. When polyglycine is precipitated from its dichloroacetic acid solution, it takes the form called polyglycine I, which is the β-form (nearly extended zigzag chain) (16). On the other hand, when polyglycine is precipitated by adding water to its aqueous LiBr solution, it takes the form of polyglycine II, which is a helical form (17).

These results suggest that glycine polymers aggregate extensively under various conditions into stable organized structures such as leaflet-like, stacked disc and sheet structures. This fluctuating system (neutral aqueous solution, 80°C) will be used for preparing various polypeptides and their molecular organizations. The formation of the organized

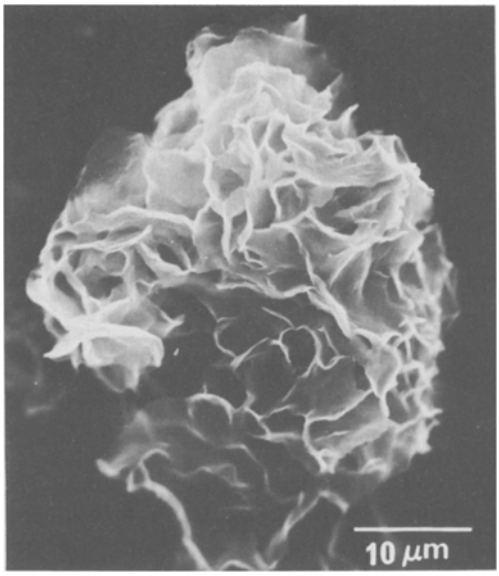

Fig. 1 Scanning electron micrograph of the glycine polymers with an average chain length of 12 which were formed from glycinamide in a fluctuating system.

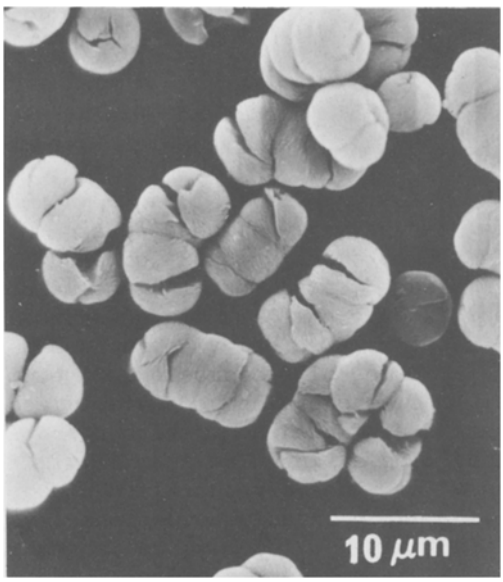

Fig. 2 Scanning electron micrograph of the organized structures produced from the LiBr solution of the glycine polymers.

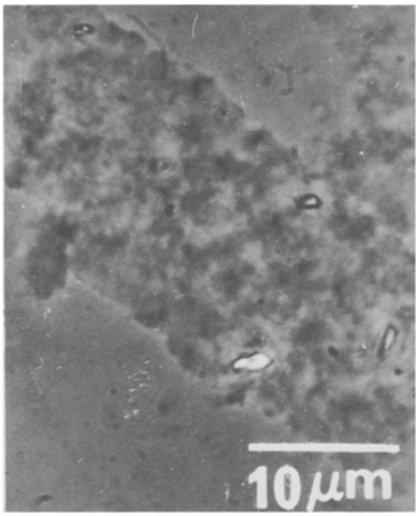

Fig. 3 Scanning electron micrograph of the organized structures produced from the dichloroacetic acid solution of the glycine polymers.

structures and enzymes from a variety of polypeptides and their physicochemical characterization will be the subject of later publications.

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