FORMATION OF PEPTIDE BONDS FROM METASTABLE VERSUS CRYSTALLINE PHASE: IMPLICATIONS FOR THE ORIGIN OF LIFE

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Abstract. Formation of peptide bonds was attempted by thermal activation of dry amino acids from aqueous solution that simulated prebiotic evaporative environments. The evaporation trend of amino acids solutions shows a bifurcation and can lead to either a crystalline phase (near equilibrium) or a metastable non-crystalline phase (far from equilibrium). Only amino acids in this metastable phase are able to form peptide bonds by thermal activation at temperatures that are generated by solar radiation today. We suggest that this metastable phase is the ideal initial material to trigger amino acid assemblage with protein-like structure because provide the driving force (supersaturation) for an intense interaction between monomers of different amino acids and allows activation of these monomers in plausible prebiotic conditions.

Keywords: metastable, crystalline, supersaturation, origin of proteins, polimerization, origin of life

1. Introduction

Peptide bonds are of paramount importance in biochemistry because they form the backbone of proteins. The activation of amino acids and formation of peptides, under primitive geological conditions remain as one of the greatest enigmas of the origin of life. Polymerization of amino acids under simulated prebiotic conditions is a well known process (Ferris *et al*., 1996; Branck, 1993) whereas thermal polymerization has been attempted as a relevant geological model (Fox and Dose, 1972; Fox and Harada, 1960). These polymers, termed thermal proteins, resemble contemporary proteins in many ways, and are regarded as models for pre-biotic proteins (Fox and Dose, 1972; Fox and Harada, 1958, 1960). Polyaminoacids have been prepared by heating of a suitable proportion of amino acids at temperatures near to 200 °C[1,2]. However, it is well known the tendency of amino acids to yield unwanted products on being heated to temperatures above the boiling point of water (Katchalsky, 1951). Furthermore, high-T conditions restrict the search of prebiotic scenarios to hot-spring ponds in volcanic environments. Dry reactant mixtures could have resulted via dissolution of amino acids in water subjected to evaporation, as would readily occur in arid regions. This scenario is simulated by initially dissolving the amino acids in water and then heating at environmentally realistic temperatures (<100 °C) (Rohlfing, 1976).

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The result of the evaporation of a poly-amino acids solution is a dry-phase of reactant mixtures, which is subsequently heated. However, which is the structural nature of this initial dry-phase? The answer depends on the initial experimental conditions.

If a solution of amino acids becomes supersaturated by evaporation, nucleation of a new phase (crystallization) can take place. The nucleation rate, from a supersaturated solution, is given by the following equation (Nielsen, 1964):

$$J = \Gamma \exp\left(\frac{-\delta\sigma^3\Omega^2}{k^3T^3(\ln S)^2}\right)$$

where δ is a shape factor, σ is the nucleus-solution interfacial tension and Ω is the volume of one growth unit in the nucleus. The pre-exponential factor Γ is related to the rate at which the nucleus can grow to a supercritical size. T is the temperature and k is Boltzmann's constant. S is the supersaturation ratio C/C_s, where C_s is the equilibrium concentration of the solution in equilibrium with the crystalline phase and C is the actual concentration of the solution.

From this expression it is clear that the nucleation rate is a direct function of supersaturation, and it is usual to define a value of the critical supersaturation as that corresponding to a nucleation rate of 1 nucleus sec^{-1} cm^{-3}. The maximum supersaturation that can be reached under given conditions is closely related to the stability of supersaturated solutions, which is governed by the so-called metastability limit.

The metastability limit marks the width of the metastable zone in which supersaturated solutions can remain without inducing crystallisation for some time. The metastability limit is a kinetic barrier for crystallization and its value will depend not only on the nature of the solute and solvent, but also on the temperature, thermal history, total mass of the solution, stirring, evaporating rate and so forth (Khamskii, 1969).

This kinetic barrier is very high for some solute molecules because the attractive forces between them are too strong for orderly crystallisation (Rosenberger *et al*., 1996), or because restrictive steric positioning required for the attachment (Wunderlich, 1976). Under these conditions a non-crystalline metastable phase may form.

It is well known that in these cases slow crystallisation from quiescent, dilute solution usually yields a metastable phase, and that a stirred solution yields crystals. It is generally accepted that this metastable phase has short-range order.

Glycine, alanine, aspartic and glutamic acids are among the most abundant amino acids formed in experiments simulating the primitive Earth. Glycine and alanine are usually formed in much larger amounts, while aspartic and glutamic acids have been found to be key amino acids for thermal copolymerization (Fox, 1995). Also, note that these four amino acids are present in the Murchison meteorite (Cronin *et al*., 1988).

On the other hand glycine, alanine, aspartic acid and serine are the repetitive sequence calculated for the original ancestral molecule of ferredoxine (Eck, 1966), which is believed to be one of the first protein formed on earth (Hall and Rao, 1971).

2. Experimental and Results

We prepared solutions (0.2 M) from doubly distillated water of each one of these amino acids (LD-Sigma) and two sets of solutions (A and B; 3%). Solution A is formed from two types of mixtures as reactants, equimolar glycine–alanine and equimolar aspartic acids–glutamic acid (10:10, 10:8, 10:6, 10:4 and 10:2 molar ratio). Solution B is formed from equimolar glycine–alanine and equimolar aspartic acid, glutamic acid and serine in the same molar ratio as A. All solutions were kept at room temperature for a few days until total evaporation.

A parallel experiment was conducted with another set of equimolar solutions A and B, which were continuously agitated during evaporation. Solutions of single amino acids totally crystallize. In both quiescent equimolar A and B solutions we observe a non-crystalline, transparent, gel-like, metastable material far from the equilibrium structure. In other mixture ratios crystalline and metastable phases coexist. As we move away from equimolar ratio the phase is more crystalline. The stirred equimolar A and B solutions crystallize totally. The metastable phase transforms spontaneously to the crystalline state by ageing the sample during a few days, at room conditions.

X-ray diffraction and polarizing microscope studies clearly show the crystalline-metastable nature of the material. Three samples of each of these products were put in an oven at 85 °C for 80 days. In a simultaneous, equivalent experiment, non-dissolved, ground crystalline equimolar mixtures of both set of A and B amino acids, were also placed in the oven. The experiments were replicated three times in successive weeks. Biuret-positive reaction test and typical thermal polypeptide infrared spectra show clearly that peptide bonds are formed in the heated metastable phase from the different molar ratio of A and B solutions (Figure 1). The absorption bands found both in acidic-basic thermal polypeptide and protein are 3300 cm^{-1}, NH stretching; 3080 cm^{-1}, NH stretching; 1650 cm^{-1}, amide I; 1550 cm^{-1}, amide II. In acid polypeptide, aspartic acid residues are involved in imide linkages when first formed. Strong absorption at 1712 cm^{-1} and mild absorption at 1780 cm^{-1} indicate the imide bond which is replaced by the peptide bond of lower wave number by gentle alkaline treatment (for more details on these typical thermal spectra see Fox and Dose (1972) and Fox and Harada (1960)). The B polymerized metastable product shows always characteristic dry bubbles that remember Fox's spherules (Fox *et al.*, 1959; Fox, 1964), although in our case the process is different, with bubbles forming spontaneously during the heating (Figure 2). No peptide bonds were detectable in any of the crystalline products.

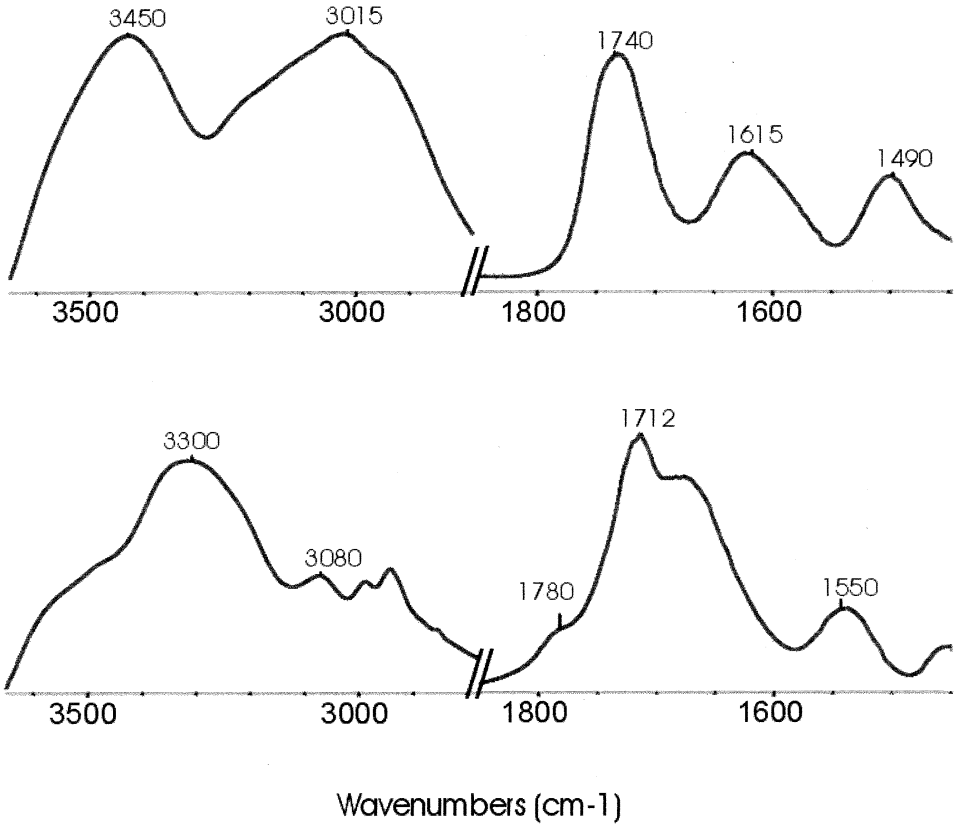

Figure 1. IR spectra of metastable phase before (above) and after heating (below).

3. Discussion

It is clear that in order to overcome the thermodynamic barrier for peptide bonding at these temperatures, a higher reactivity of monomers than in the crystalline state (intra-crystal or crystal–crystal molecular interaction) is necessary. This high reactivity is only found in the far from equilibrium metastable state (Figure 3). Thus, formation of a metastable phase is the only way by which molecules of different amino acids, specially between amino acids with little degree of isomorphism, can order together like a protein sequence at high supersaturation levels.

To avoid crystallization a solution needs special kinetic and compositional characteristics. If a solution shows fractional crystallization and both crystalline and metastable phases appear, only those amino acids molecules that have incorporated to the metastable phase are able to polymerize.

This might prove to be a good selective process for more complicated mixtures of amino acids or other molecules. On the other hand Kondepudi and Nelson (1985), and Avetisov *et al.* (1991) stated that the kinetic behavior of hypothetical

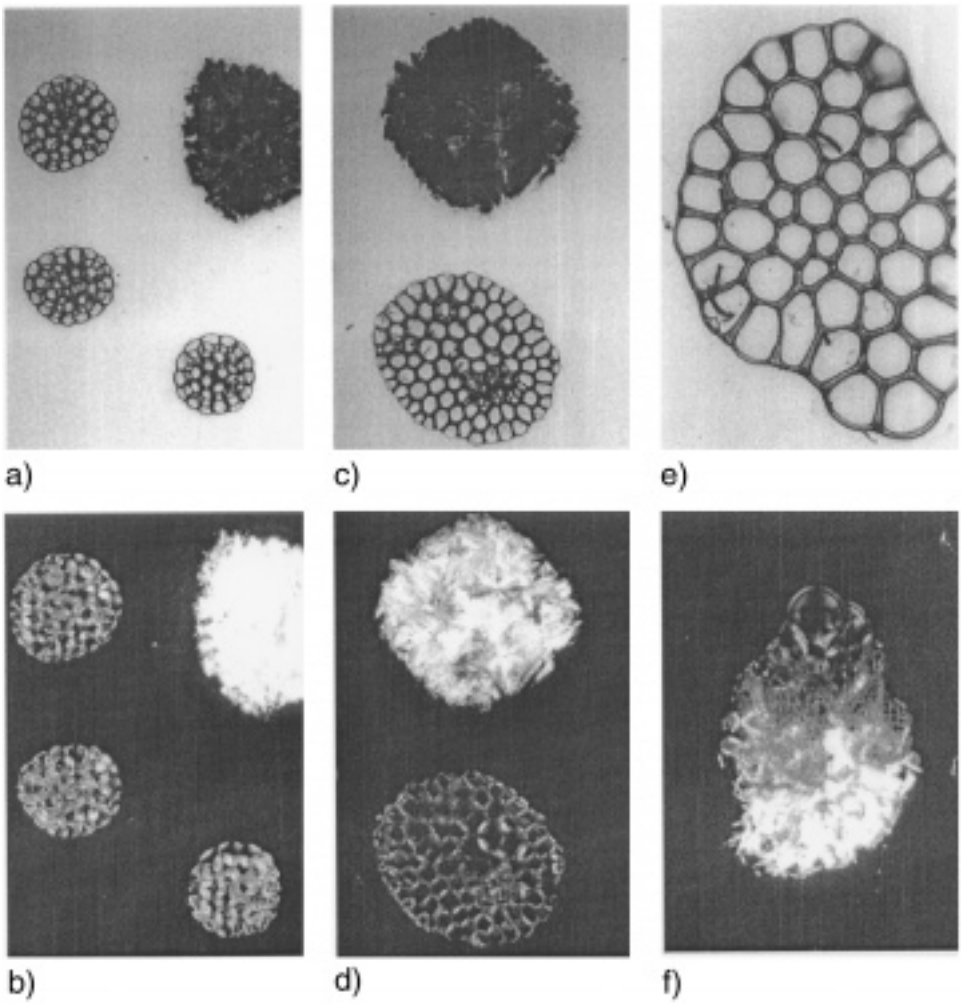

Figure 2. Optical microphotographs (×12) of a set of drops from the same solution of amino acids B after evaporation and ageing for a few days. During the ageing process we selected some crystallised drops, some metastable drops and another one partially metastable-crystalline drop. All them were heated at 85 °C for 80 days. Only amino acids in metastable state formed peptide bonds. Microphotographs **a** and **c** with transmitted illumination show metastable original drops with curious dry empty bubbles formed during the heating time and with peptide bonds. The other two crystalline drops kept without morphological change and no peptide bonds were detectable. Microphotographs **b** and **d** are the same samples viewed through crossed polarizers showing the crystalline nature of the drops with no peptide bonds (interference colours) and the non-crystalline nature of the bubble-drops with peptide bonds. Microphotograph **e**: 2-D view of the interaction between different bubbles with heptagonal, hexagonal and pentagonal perimeters. Microphotograph **f** shows an originally partially metastable-crystalline drop between crossed polarizers. It is easy distinguish both phases after heating. Down crystal material with interference colours and no peptide bonds. Top typical bubbles with peptide bonds.

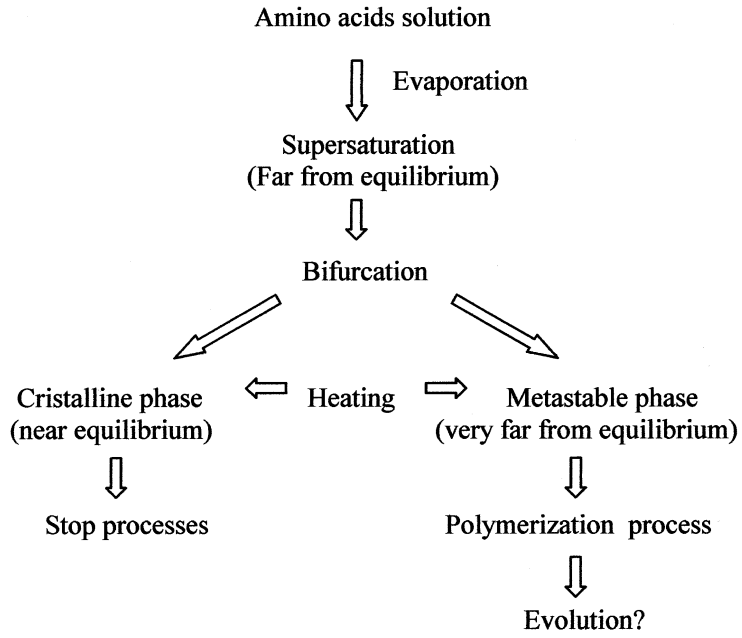

Figure 3. Schematic diagram showing the two pathways that amino acids follow in our experiments, with a speculative end. Depending on experimental conditions when a solution of amino acids evaporate totally, amino acids appear either crystalline or metastable state. Heating both at temperatures within the range of those that are generated by solar radiation today, only amino acids in metastable state form peptide bonds.

unstable, far from equilibrium, racemic system which pass through a bifurcation point may randomly divaricate into a condition of enantiomeric purity, because, as Nicolis and Prigogine have proposed, such bifurcations far from equilibrium endow the system with a pronounced sensitivity to slightest factors which might induce the non-random selection of a preferred molecular chirality. We believe that the small differences in physical properties, equilibria and reaction rates for enantiomers caused by parity-violating energy differences theory (PVEDs), may influence in the crystallization process of our racemic far from equilibrium metastable system. A hypothetical non-random crystal-molecular chirality would mean a non-random peptide-molecular chirality. This work is in progress.

It is known that amino acids that do not thermally polymerize alone do so together (Harada and Fox, 1958; Vegotsky *et al.*, 1958). In the same way, as we show here, amino acids that crystallize from solution alone keep as a metastable phase together. We believe that both cases might be related.

Temperatures tested here are within the range of those that are generated by solar radiation today in some terrestrial localities (Rohlfing, 1976) and, as it is well known (Rohlfing, 1976; Fox, 1995), time can be traded for temperature. We must conclude, therefore, that the heating of a metastable phase previously formed at

these moderate temperatures, is the simplest mechanism by which peptides could have formed on the primitive Earth. Thus the evaporating lagoon scenario represents a potentially interesting model for thermal polymerization.

Finally, we agree with Ferris (1989) that more selective conditions than those used in classic thermal polymerization should be set up in the experimental work aimed to obtain self-ordering in prebiotic peptide formation. For example, temperatures in the order of 200 °C followed by fast cooling may yield misleading results. Thus, here we suggest an alternative, more 'realistic' (probable T conditions on early Earth), and 'selective' approach to attain self-ordering in peptides in the reaction products. We may accomplish this in terms of the interaction between the following mechanisms: 1) slow formation process at room temperature of a metastable phase; 2) high supersaturation levels and therefore, intense intermolecular interactions; and 3) probable restrictive steric positionings required for the attachment of different amino acids molecules in the metastable phase.

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