ABIOTIC ORIGIN OF BIOPOLYMERS*

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Abstract. A variety of methods have been investigated in different laboratories for the polymerization of amino acids and nucleotides under abiotic conditions. They include (1) thermal polymerization, (2) direct polymerization of certain amino acid nitriles, amides or esters, (3) polymerization using polyphosphate esters, (4) polymerization under aqueous or drying conditions at moderate temperatures using a variety of simple catalysts or condensing agents like cyanamide, dicyandiamide, imidazole, etc., and (5) polymerization under similar mild conditions but employing activated monomers or abiotically synthesized high energy compounds such as adenosine 5'-triphosphate (ATP). The role and significance of these methods for the synthesis of oligopeptides and oligonucleotides under possible primitive Earth conditions is evaluated. It is concluded that the latter more recent approach involving chemical processes similar to those used by contemporary living organisms, appears to offer a reasonable solution to the prebiotic synthesis of these biopolymers.

1. Introduction

The field of science devoted to the study of chemical evolution and the origin of life dates back to Oparin's hypothesis of fifty years ago (Oparin, 1924) when, with great insight, he postulated that life must have originated on the primitive Earth from a pool of organic chemicals under reducing conditions. It is therefore an honor to be invited to contribute a paper on the abiotic origin of biopolymers in the International Seminar commemorating the publication of Oparin's book *The Origin of Life*. We have recently published two reviews related to this topic (Stephen-Sherwood and Oró, 1973; Oró and Stephen-Sherwood, 1974) and will therefore only try to highlight current trends in this field, minimizing as far as possible repetition of work described in these two previous papers.

In order to understand the abiotic origin of oligopeptides and oligonucleotides, it is essential to have some knowledge of the environmental conditions which led to their abiogenesis. The discovery of a number of simple organic molecules in interstellar space, comets and meteorites has left little doubt as to the universality of organic cosmochemistry and the reducing conditions assumed to have prevailed on the primitive Earth (Oró, 1972). It is significant that five of the interstellar molecules, ammonia, water, hydrogen cyanide, formaldehyde and cyanacetylene, together with H_2, CH_4, etc., which are known to be present in the atmospheres of the Jovian planets, have been shown through chemical experimentation to be important precursors in the abiotic formation of amino acids, sugars, purines and pyrimidines. If phosphate and simple salts are included, the list of precursors for the chemical synthesis of the building blocks of nucleic acids and proteins is essentially complete. As a result of the action of various forms of energy (electric discharge, cosmic rays, ultraviolet light, heat, etc.) on these simple precursors, a highly complex system of organic chemicals would probably have developed in the lakes and ponds

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of the primitive Earth. Such a complex system of organic molecules has been postulated as a prerequisite to the evolution of life (Oparin, 1924).

Once a self-replicating system, or proto-organism developed, then of necessity, the chemical environment would change as a result of biological activity. It has been suggested (Horowitz, 1945) that some of the biological pathways of modern organisms developed as a result of a changing chemical environment. As a corollary to this hypothesis, we can conclude that some of the present day biological pathways are a modified blue-print of their chemical origins. There is some indication of the validity of this proposal. The intermediates in the abiotic synthesis of adenine, e.g., 4-amino-5-imidazole carboxamide (Oró, 1960; Oró and Kimball, 1961) and also thymine (Stephen-Sherwood *et al.*, 1971) are analogs of the compounds involved, e.g.: 4-amino-5-imidazole carboxamide ribotide, in the corresponding biosynthetic reactions occurring in living organisms. We must stress that this type of argument does not take into account changes which must have occurred in biological processes during subsequent evolution, and that therefore a correlation between biological and prebiotic pathways may only be valid in a limited way. However, a direct correlation between these two pathways may still be evident in some of the major synthetic reactions. With this in mind we would like to suggest as a working hypothesis that the major biosynthetic pathways are a recapitulation of the most efficient prebiotic reactions which ocurred under physical conditions presumably not greatly different from the ones prevailing in primitive organisms. Therefore, it may be expected that positive correlations will be found not only in the formation of some essential monomers but also in the formation of the major biopolymers.

2. Abiotic Synthesis of Biopolymers

Several procedures have been studied by different investigators for the polymerization of amino acids and nucleotides under abiotic conditions. They include (1) thermal polymerization, (2) direct polymerization of certain amino acid precursors such as amino acid nitriles, amides or esters, (3) polymerization using polyphosphate esters, (4) polymerization under aqueous or drying conditions at moderate temperatures using a variety of simple catalysts or condensing agents like cyanamide, dicyandiamide, imidazole, etc., and (5) polymerization under similar mild conditions but employing activated monomers or abiotically synthesized high energy compounds such as aminoacyl adenylates and nucleoside 5′-triphosphates. This latter more recent approach involves chemical processes similar to those used by contemporary living organisms, and may offer a reasonable solution to the early prebiotic synthesis of these biopolymers. The majority of these methods are applicable to the synthesis of both biopolymers. We will therefore consider their formation consecutively under the above five headings. As both polypeptide and polynucleotides probably evolved under similar conditions on the primitive Earth, such an analysis will help to evaluate their mode of abiogenesis.

2.1. Thermal polymerization

The thermal condensation of dry mixtures of amino acids by heating to temperatures of 150°–180°C yield protein-like material (proteinoids) (Fox and Middlebrook,

1954; Harada, 1959; Harada and Fox, 1958, 1965). Copolymers which contain 18 of the amino acids commonly found in proteins have been produced (Fox and Harada, 1958, 1960, 1963). Various proteinoids have now been synthesized which will catalyze hydrolytic (Rohlfing and Fox, 1969), decarboxylation (Rohlfing, 1967), amination (Krampitz *et al.*, 1968a), deamination (Krampitz *et al.*, 1968b), oxidoreduction (Dose and Zaki, 1971) and polymerization (Fox *et al.*, 1974) reactions. The activity of the proteinoids does not decrease with storage over a period of 5-10 years (Rohlfing, 1970).

Under similar anhydrous conditions, uridine and uridine 2′(3′)-phosphate condense to yield di- and trinucleotides in yields of 20% and 7% respectively. These oligomers contain only [2′ → 5′]- and [3′ → 5′]-internucleotide bonds, with a preference for the natural [3′ → 5′]-linkage (Morávek *et al.*, 1968a, b). The heating of uridine 2′(3′)-phosphate to 160°C yields dinucleoside diphosphates and trinucleoside triphosphates of uridine, in yields of 23% and 12% respectively, containing predominantly [3′ → 5′]-internucleotide bonds in addition to [2′ → 5′]-bonds (Morávek *et al.*, 1968c). The polymerization of the triethylammonium salt of cytidine 2′ : 3′-cyclic phosphate at 130°C for 48 hr., produced oligomers of cytidylic acid up to the hexamer, consisting of both [3′ → 5′]- and [2′ → 5′]-phosphodiester bonds. Approximately 50% of the dimer had the natural linkage (Tapiero and Nagyvary, 1971).

The temperatures employed in these condensations are too high to have been of general occurrence on the primitive Earth. However, in area of volcanic activity such temperatures may have occurred under special conditions, and it is significant that some of the polymeric products formed under these conditions have catalytic activity.

2.2. Direct polymerization of amino acid precursors

Polymers of amino acids have been detected among the products of electric discharge experiments (Miller, 1955; Oró; 1963a; Grossenbacher and Knight, 1965; Matthews and Moser, 1966). The polymeric material obtained in the polymerization of HCN under ammoniacal conditions may be converted to amino acids upon hydrolysis (Oró and Kamat, 1961; Lowe *et al.*, 1963; Matthews and Moser, 1966, 1967). It remains to be determined the extent to which peptide bonds are present in these polymers.

On the other hand it is known that peptides are formed from nitriles, amides and other precursors. Thus, when amino-acetonitrile is heated with kaolinite at 130-135°C peptides of glycine are formed (Akabori, 1959). When glycinamide is heated in aqueous solution at 100°C, polyglycine is formed. Infrared and X-ray diffraction data showed that the homopolypeptide corresponded exactly with polyglycines I and II (Oró and Guidry, 1960). The polymerization of glycine in aqueous ammonia solutions may also occur through formation of glycinamide (Oró and Guidry, 1961). Aspartyl peptides are formed by heating asparagine in water at 70–75° or 100°C (Kovacs and Nagy, 1961). Serine and threonine-containing heteropolypeptides were prepared by the addition of formaldehyde and acetaldehyde on polyglycine (Akabori, 1959).

2.3. Polymerization using polyphosphate esters

Ethyl polyphosphate esters, which are probably mixtures of cyclic metaphosphate and straight chain pyrophosphate esters, promote the condensation of both amino acids and nucleotides. Polyarginine, of molecular weight 4000–5000, was synthesized by heating arginine with polymetaphosphate ester (Schramm *et al.*, 1962). Homopolymers of DL-leucine, DL-phenylalanine, DL-serine and DL-valine have been prepared in yields of 13–57% by heating the above amino acids with polymetaphosphate ester to 60°C for 10–24 hr (Nooner and Oró, 1974). The degree of polymerization was about 12 amino acid units for polyleucine. Copolymers were also prepared but in lower yield (4–5%). Di- and tri-peptides of glycine and alanine have been obtained in dilute, neutral or slightly alkaline (pH 7.5–9.5) solutions in the presence of linear polyphosphates or trimetaphosphate (Rabinowitz *et al.*, 1969; Rabinowitz, 1969). Glycine reacts initially with trimetaphosphate to form a cyclic acylphosphoramidate and pyrophosphate (Chung *et al.*, 1971). The cyclic compound then reacts with the free amino group of glycine or diglycine to give diglycine-N-phosphate or triglycine-N-phosphate. The protection of the amino group by phosphorylation prevents the formation of higher oligopeptides.

Polynucleotides of high molecular weight are formed when ribonucleotides are heated with ethylpolyphosphate esters (Schramm *et al.*, 1962; Kochetkov *et al.*, 1964; Schramm, 1965). Subsequent work has shown that these polyribonucleotides has branched chains (Gottich and Slutsky, 1964). Although the significance of these nonaqueous reactions to prebiological organic chemistry is questionable, they do indicate that polyphosphates may have played an important role in abiotic condensation reactions. This topic will be discussed in greater detail later. Polyphosphates may be synthesized under primitive earth conditions by heating ammonium phosphate with urea. Addition of a nucleoside (deoxythymidine or dideoxythymidine) to the system promotes trimetaphosphate synthesis (Osterberg and Orgel, 1972).

2.4. Polymerization under aqueous or drying conditions at moderate temperatures using a variety of catalysts or condensing agents

Cyanamide (Oró, 1963b) and dicyanamide (Steinman *et al.*, 1965a) are considered plausible condensing agents for use in primitive Earth experiments. Under mild aqueous conditions, cyanamide (Ponnamperuma and Peterson, 1964), dicycanamide (Steinman *et al.*, 1965a), dicyanadiamide (Steinman *et al.*, 1965b), and the hydrogen cyanide tetramer, diaminomaleonitrile (Chang *et al.*, 1969) will condense amino acids to low molecular weight peptides. Also, cyanate has been found to increase several fold the yields of glycine peptides obtained by heating glycine and apatite, or some orthophosphates at 95°C (Flores and Leckie, 1973).

Low yields of oligodeoxynucleotides were obtained when deoxythymidine 5′-phosphate was heated in aqueous solution with cyanamide (Ibanez *et al.*, 1971b and c). Imidazole also promotes the condensation of deoxythymidylic acid under aqueous conditions, oligomers of up to five units were obtained containing mainly [3′ → 5′]-phosphodiester bonds in yields of less than 1% (Ibanez *et al.*, 1971a). As these aqueous polymerization reactions have met with only limited success, Orgel and coworkers have employed a procedure, which has proved successful, in the abiotic

synthesis of purine nucleosides and abiotic phosphorylation reactions, and for the condensation of ribonucleotides. This procedure simulates the drying of primordial lakes and ponds, and therefore involves a reaction involving moderate temperatures (below 100°C) and ambient humidity. Oligonucleotides are formed when a uridine, urea, ammonium dihydrogen phosphate system is heated at temperatures from 85°C to 100°C. Di- and trinucleotides were formed in good yields, with a preference for the natural [3′ → 5′]-phosphodiester bond (Osterberg *et al.*, 1973). Under similar conditions, adenosine cyclic 2′, 3′-phosphate polymerizes to give high yields of oligonucleotides of up to six units in length, when heated (25–85°C) under alkaline conditions in the presence of aliphatic diamines. The oligomers showed a preference for the natural [3′ → 5′]- over a [2′ → 5′]-linkage (Verlander *et al.*, 1973). Efficient phosphorylations of uridine (Schwartz, 1972) and deoxythymidine (Schwartz, 1973) have also been accomplished with a system involving either apatite and ammonium oxalate with one of several condensing agents (e.g., cyanamide, dicyandiamide, urea) or with only apatite (hydroxyl- or fluoroapatite) and cyanogen water mixtures. Apparently cyanogen does not act directly but after it has been transformed into urea and ammonium oxalate. However, aside from the nucleoside monophosphates, the more highly charged products synthesized by this procedure were not adequately analyzed to determine their degree of polymerization.

2.5. Polymerization under mild conditions using activated monomers

As the preceding discussion has shown, unless drastic conditions, like thermal polymerization, are employed, the yields and degree of polymerization of both polypeptides and oligonucleotides in general are low. Recent work from a number of laboratories has therefore been directed toward investigating the use of activated monomers in abiotic polymerization reactions.

A number of activating groups could have been of importance on the primitive Earth. These include polyphosphates (nucleoside triphosphates, as well as linear polyphosphates and trimetaphosphate), phosphoramidates, acylphosphates and phosphorimidazolides. Any student of contemporary biochemistry is familar with the vital role that some of these compounds (e.g. nucleoside triphosphates) play in the metabolism of living organisms. The synthesis of ribonucleoside triphosphates (Neuman *et al.*, 1970), deoxyribonucleoside triphosphates (Joe and Oró, 1975), linear polyphosphates and trimetaphosphate (Osterberg and Orgel, 1972), phosphoramidates (Lohrmann and Orgel, 1973), acylphosphates (Lowenstein and Schatz, 1961) and phosphorimidazolides (Lohrmann and Orgel, 1973) have all been reported under primitive Earth conditions.

Nucleoside di- and triphosphates have also been synthesized with the help of ethylpolymetaphosphate and ultraviolet light (Ponnamperuma, 1965) or by the action of polyphosphoric acid at low temperatures (Waehneldt and Fox, 1967). However, as in the work of Schramm *et al.* (1962), the significance of these experiments to prebiotic synthesis is questionable because the phosphate derivatives used are not considered geologically relevant.

An example of the application of the approach of using activated monomers to the synthesis of polypeptides is the condensation of aminoacyl adenylates, in

slightly alkaline solution pH (7.8–8.5) at room temperature, which yields polypeptides of six units or more (Paecht-Horowitz and Katchalsky, 1967; Lewinsohn *et al.*, 1967). In the presence of montmorillonite, which absorbs aminoacyl adenylates, but not amino acids or adenylic acid, the degree of polymerization was as much as 54 peptide units. A high percentage of the peptides were attached through an ester linkage to the 2′ or 3′ OH group of adenylic acid (Paecht-Horowitz *et al.*, 1970; Paecht-Horowitz, 1971 and 1974). Aminoacyl adenylates are reported to be formed when an amino acid and ATP are absorbed by an ion exchanger, zeolite 'Decalso F', a synthetic equivalent to mineral alumino silicates (Paecht-Horowitz and Katchalsky, 1973), however workers from another laboratory have not been able to confirm this result (Warden *et al.*, 1974).

If an amino acid, like glycine, is heated (65° to 100°C) under conditions of ambient humidity with ATP and magnesium ions, a phosphoramidate is formed. Though phosphoramidates do not polymerize on heating alone, if an excess of imidazole is added to the reaction di- and short peptides are formed in yields of up to 10% (Lohrmann and Orgel, 1973). Even higher yields are obtained by heating glycine with the adenosine 5′-phosphorimidazolide. This imidazole derivative, like phosphoramidates, is obtained in low yield by heating imidazole with ATP in the presence of magnesium ions. When the N-protected methyl ester of alanine is heated with glycine in the presence of excess imidazole, alanyl-glycine is formed (Wieland and Vogeler, 1963). It should be noted that the phosphoramidate solid state synthesis of peptides, although informative for an understanding of possible prebiotic mechanisms of synthesis, has little in common with contemporary biochemical processes (Lohrmann and Orgel, 1973).

Dipeptides are formed in 5–10% yield when amino acids such as glycine or phenylalanine are heated at 90°C under conditions of ambient humidity in the presence of cyanamide, 4-amino-5-imidazolecarboxamide (AICA) and ATP. In the absence of ATP or AICA, the yield of peptide is much reduced, when cyanamide is absent no peptide is formed at all (Stephen-Sherwood *et al.*, 1974a). Work is in progress to determine whether higher peptides are also formed.

In a comparable experiment to the one we used for synthesizing dipeptides, we have found that when deoxythymidine 5′-triphosphate is heated in the presence of cyanamide, AICA and dTMP, oligonucleotides of the formula (pT)n, $n = 2$–4 are formed in good yields (Stephen-Sherwood *et al.*, 1974b). The yields of oligonucleotides are reduced when AICA is absent, and no oligonucleotides are formed when cyanamide is omitted from the reaction mixture. Cyanamide is a well accepted abiotic condensing agent and appears to be an essential ingredient in the above abiotic polymerization reactions.

If any one molecule, in present day biological systems, were to be assigned the role of being the key to living processes, that molecule would probably be ATP. It is not surprising therefore that recent experimental work from several laboratories, including our own, has demonstrated the importance of ATP and other nucleoside triphosphates (e.g. dTTP) in the abiotic synthesis of both polypeptides and oligonucleotides under possible primitive Earth conditions. It has been suggested (Lohrmann and Orgel, 1973) that ATP condenses initially with imidazole to form adenosine 5-phosphorimidazolide which functions as a key intermediate in the

activation of amino acids and nucleotides for abiotic polypeptide and oligonucleotide formation. The formation of such an intermediate implies that the catalytic role of imidazole in these abiotic polymerization reactions is that of a nucleophile.

Alternative mechanisms should also be considered. Pong and T'so (1969, 1971) have suggested that in the polymerization of dTMP in N, N-dimethylformamide, β-imidazole 4(5)-propionic acid functions as proton donor, and that a pyrophosphate, P^1, P^2-dideoxythymidine 5'-pyrophosphate is an intermediate in oligonucleotide formation. Triethylamine hydrochloride also catalyzes this polymerization reaction. If the catalytic role of imidazole or its derivatives is mainly that of a proton donor, a mechanism analogous to that proposed by Ibanez *et al.* (1971a) could explain the polymerization of thymidine 5'-triphosphate to oligonucleotides under aqueous or drying conditions (Figure 1). Yet another possibility (Oró and Stephen-Sherwood, 1974; Stephen-Sherwood *et al.*, 1974b) is that a cyclic trimetaphosphate derivative is initially formed when a nucleoside 5'-triphosphate is heated under conditions of ambient humidity. Nucleoside trimetaphosphates could function as activated intermediates in polypeptide and oligonucleotide synthesis in reactions conducted under drying conditions, but would be unstable in aqueous solutions. Inorganic polyphosphates have been shown to cyclize under comparable conditions (Osterberg and Orgel, 1972). It is also known that cyclic alkyl-trimetaphosphates have been postulated as one active phosphorylating species, in the condensation of nucleotides in pyridine using dicyclohexylcarbodiimide (DCC) as a condensing agent (Weiman and Khorana, 1962; Jacob and Khorana, 1964).

The above mechanism involving a cyclic trimetaphosphate intermediate has received strong support by the recent detection by NMR of adenosine 5'-trimetaphosphate in the DCC mediated condensation of ATP (Glonek *et al.*, 1974). Whatever the mechanism of these abiotic polymerization reactions is, the importance of

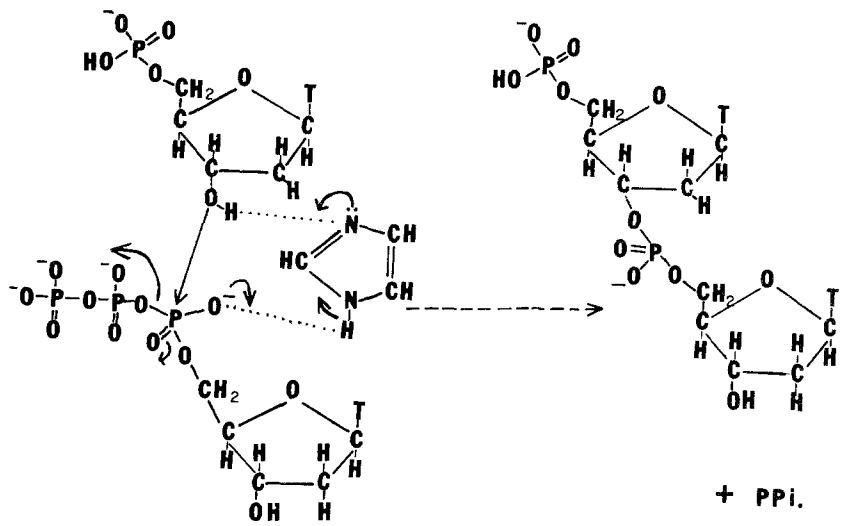

Fig. 1. A mechanism for the polymerization deoxythymidine 5'-triphosphate, using imidazole or an imidazole derivative as catalyst.

both polyphosphates and imidazoles is evident. Imidazole is formed under primitive earth conditions by irradiation of the adduct of ammonia and cyanacetylene with ultraviolet light (Ferris *et al.*, 1968). The imidazole derivative, AICA, would also have been readily available in the abiotic milieu as a result of the hydrolysis of 4-amino-5-imidazole carbonitrile, an intermediate in the synthesis of adenine (Oró and Kimball, 1961; Ferris and Orgel, 1966). The reactions described in this section resemble the contemporary biochemical pathways used in nucleic acid and protein biosynthesis insofar as similar intermediates are involved in both the chemical and biological processes.

3. Discussion

Through syntheses described in the previous sections it is feasible that short chain polypeptides and oligonucleotides would be present in the prebiotic soup. It is unlikely that the oligonucleotides formed would have been of sufficient length of code for polypeptides with catalytic properties. The emergence of a nucleic acid mediated protein synthesizing system probably occurred at some later stage during chemical evolution. The length of a nucleotide chain required to serve as a primer in chemical replication is a matter of speculation, but based on the evidence of enzymatic synthesis using short chain oligonucleotide primers (Kornberg *et al.*, 1964; Wells *et al.*, 1967a; Wells *et al.*, 1967b) and molecular hybridization studies on natural polynucleotides (Walker, 1969; Niyogi and Thomas, 1967; Ruger and Bautz, 1968), oligonucleotides of about 12 units or more could have formed suitable templates for a self-replicating system. In an earlier paper (Oró and Stephen-Sherwood, 1974) we have suggested two models to account for the replication and elongation of short oligonucleotides. One model is based on a slippage mechanism originally proposed by Chamberlain and Berg (1962) to account for the enzymatic synthesis of high molecular weight oligomers using short chain primers. The second model utilizes a mechanism, similar to the rolling circle model devised to explain the replication of circular DNA in many procaryotic cells (Gilbert and Dressler, 1968), and envisages a circular template with a region of limited stacking on which free nucleotides would stack, hydrogen bond, and polymerize.

The mechanism for abiotic nucleic acid replication is at best poorly understood. Adenosine cyclic 2′, 3′-phosphate polymerizes on a polyuridylic acid template in the presence of polybasic amines or glycine derivatives to yield di- and tri-nucleotides with mainly [2′ → 5′]-bonds (Renz *et al.*, 1971). Adenosine 5′-triphosphate forms a stable helix with polyuridylic acid (Miles *et al.*, 1966) but undergoes hydrolysis without appreciable oligonucleotide formation (Weimann *et al.*, 1968). Adenosine 5′-phosphoimidazolide reacts on a polyuridylic acid template to form di- and trinucleotides in yields of 43.5% and 2.8%, respectively, but the major dinucleotide formed had [2′ → 5′]-phosphodiester bonds (Weimann *et al.*, 1968). Deoxyadenyl (3′-5′)-deoxyadenyl-(5′-N)-phenylalanine condenses on a polyuridylic acid template to yield higher oligomers (Shabarova and Prokofiev, 1970). It is evident that some catalyzing factor is missing from the systems so far investigated. This factor may have been a simple polypeptide containing amino acids like lysine, histidine or arginine. Work

is in progress to determine the effects of such compounds, as well as of cyanamide-imidazole derivative pairs, on the synthesis and replication of oligonucleotides.

The effects of different types of radiation, such as UV and ionizing radiation, on the abiotic synthesis of biopolymers were not discussed here since they were considered beyond the scope of this review.

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