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Novel Prebiotic Systems: Nucleotide Oligomerization in Surfactant Entrapped Water Pools

D.W. Armstrong[1], F. Nome[1], J.H. Fendler[1]*, and J. Nagyvary[2]

[1] Department of Chemistry and
[2] Department of Biochemistry
and Biophysics, Texas A & M University, College Station, Texas 77843, USA

Summary. Oligomerization of 5′-TMP in water pools entrapped by dodecyl-ammonium chloride surfactant aggregates in benzene : hexane in the presence of dicyanodiimide at temperatures ranging from 21°–72° resulted in the formation of linear and cyclic oligonucleotides containing up to pentamers. Effects of temperature, time and surfactants have been examined. Rate constants for the formation of oligomers have been determined at five different temperatures. These data afforded values of $\Delta H^{\ddagger} = 11.8 \pm 1.9$ Kcal mole^{-1}, $\Delta S^{\ddagger} = -53 \pm 6$ e.u. and $\Delta G^{\ddagger} = 27.4 \pm 4.0$ Kcal mole^{-1}. Prebiotic significance of these results are discussed.

Key words: Dicyanodiimide – Nucleotide polymerization – Reversed micelles – Compartmentalization – Thermodynamics of nucleotide polymerization

Introduction

The demonstration of an efficient nucleotide polymerization under plausible prebiotic conditions is an important and essential requirement for the substantiation of any proposal concerning macromolecular evolution. The role of colloidal systems in prebiotic chemistry has been implicated at different times (Herrera, 1942; Oparin, 1957, 1965). We have proposed a physical-chemical model for the primitive codon assignments (Nagyvary and Fendler, 1973, 1974, 1976). The essential requirement of this model is that the two protagonists preferentially meet each other. This is accomplished in micellar compartments into which nucleotides and amino acids are sequestered by virtue of suitable discriminating criteria. Formation of micelles from relatively simple surfactants in water and in nonpolar solvents is well documented (Fendler and Fendler, 1975; Fendler, 1976) and their postulated prebiotic presence is based upon the suggested composition of the primordial ocean (Lasaga et al., 1971). Solubility and dipole-dipole interactions are the most obvious criteria for sequestering amino acids and nucleotides into micellar

* To whom offprint requests should be sent

compartments. Indeed, we demonstrated differential partitioning of cationic amino acids, arginine and lysine, into aqueous micellar sodium dodecanoate compartments. Conversely, uptake of serine, glycine, aspartic acid, glutamic acid, threonine, alanine, proline, valine, leucine, phenylalanine and isoleucine into this compartment is indiscriminate (Fendler et al., 1975). Differential electrostatic interactions have been observed, however, in the binding of adenosine, guanosine, cytidine, uridine, AMP, GMP, CMP, and UMP to the same micellar compartment (Nagyvary et al., 1976).

Amino acid uptake into surfactant solubilized water pools in reversed micelles is dependent on both electrostatic and hydrophobic interactions (Fendler et al., 1975). This water pool provides unique catalytic media and can be considered to be a simple model for the cell interior (Fendler, 1976). Additionally, reversed micelle forming alkyl ammonium surfactants bind strongly to polynucleotides (Dutta et al., 1953; Khym and Uziel, 1968). Results are presented in this paper for enhanced oligonucleotide formation in surfactant solubilized water pools using only "accepted prebiotic reagents". Furthermore, oligomerization has been observed in this system at 21 °C and at 5′-thymidylic acid concentrations as low as 1.0×10^{-5} M.

Experimental Procedure

The best available grade 5′-thymidylic acid (TMP), 3′,5′-cyclic thymidylic acid (cyclic TMP), and thymidine were purchased from Sigma Chemical Company. No impurities were detected by absorption spectrophotometry and by descending paper chromatography. Reagents were, therefore, used as received. The enzymes used for chain length analysis, alkaline phosphatase and snake venom phosphodiesterase were also obtained from Sigma as was the DEAE cellulose and the dicyclohexylcarbodiimide (DCC). Dicyanodiimide (cyanoguanidine) was obtained from Matheson, Coleman & Bell Co. and used as received (no impurities were detected with IR and NMR spectroscopy). Stock solutions of hexane and benzene were distilled from sodium and stored over Linde Type 5A molecular sieve. Sodium dodecyl sulfate (SDS, 99.9 % pure) was the generous gift of Kao Soap Co. (Tokyo, Japan). Hexadecyltrimethylammonium bromide (CTAB), Sigma and sodium dodecanoate, Aldrich Gold Label, were dried in vacuo for 48 h over P_2O_5 prior to use. Dodecylammonium propionate (DAP) was prepared by the established method (Fendler et al., 1972). Lauroyl histidine was prepared by the method of Gitler and Ochoa-Solano (1968). Dodecylammonium chloride (DACl) was prepared by bubbling HCl gas into a two molar solution of dodecylamine in diethyl ether. After 20 min the precipitated dodecylammonium chloride was filtered and thoroughly washed with fresh diethyl ether. The precipitate was then recrystallized three times from benzene and dried in vacuo over P_2O_5; mp, 182.5–184 °C.

In a typical experiment 19.3 mg. (3×10^{-3} M) of 5′-TMP and 200 mg. of dicyanodiimide, a recognized prebiotic dehydrating agent (Steinman et al., 1964; Ponnamperuma and Peterson, 1965; Calvin 1969), were place in a round bottom flask with 10.0 ml. hexane and 10.0 ml. of benzene. 9×10^{-4} moles of surfactant were added to the reaction mixture. The flask, fitted with a reflux condenser and drying tube, was then placed in a thermostated oil bath and the reaction was stirred continuously. In different experiments, the temperature, reaction time, and surfactant were varied. The water con-

tent of the reactions was adjusted to 0.01 M and monitored by gas-liquid partition chromatography as previously described (Fendler and Liu, 1975).

Upon completion, the reaction mixture was rotary evaporated to dryness. The white to beige colored powder was then treated with acetic anhydride to remove any pyrophosphate (Khorana et al., 1962). The mixture was again evaporated to dryness after which 3.0 ml. of water ($\leq 1\ ^{\circ}C$) was added. The water mixture was left for 30 min at $\leq 1\ ^{\circ}C$, with intermittent shaking. The solution was then gravity filtered at $\leq 1\ ^{\circ}C$ to remove any insoluble material. The water soluble material containing the oligomers of TMP were separated on a DEAE cellulose column (60.0 cm. long, 2.0 cm. diameter) with a linear triethylammonium bicarbonate (pH = 7.5) gradient. The peaks were detected at 267 nm using a Cary 118C spectrophotometer. R values for the oligomers were obtained using descending chromatography (Whatman 1 chromatographic paper) in 2 different solvent systems. Solvent A was 7 : 1 : 2, isopropanol : concentrated NH_4OH : H_2O by volume. Solvent B was 100 : 60 : 1.6, isobutyric acid : 1 N NH_4OH : 0.1 M EDTA disodium salt by volume. The chain length of the linear dimer and trimer (see Results) was confirmed by enzyme degradation (Khorana and Vizsolyi, 1961). Terminal phosphomonoester groups were removed with alkaline phosphatase. The reaction mixture contained 10.0 optical density units of oligomer at 267 nm, 5 μl of 100 units/ml. enzyme solution in 0.25 ml. of 0.05 M Tris HCl buffer pH = 8.0. The mixture was incubated at 37 °C for 30 min. Removal of the 5′ phosphate group was complete in all cases and the activity of the enzyme was monitored with p-nitrophenyl phosphate and 5′-TMP standards. The products were separated using solvent A. Cleavage of the 3′→5′ oligonucleotide bond was done using snake venom phosphodiesterase as described by Sulston et al. (1968). The degradation was done in 0.25 ml. of 0.10 M Tris HCl buffer, pH = 9.0, with 2.0 optical density units of oligomer at 267 nm and 0.10 units of enzyme. The solution was incubated at 37 °C for 30 min and the products were separated using solution A. Cyclic oligonucleotides were also confirmed by the fact that they were not affected by alkaline phosphatase.

The percent yields shown in Tables 3, 4 and 5 were calculated as follows:

$$\% \text{ Yield} = \frac{\text{O.D. 267-units (oligomer)}}{\text{O.D. 267-units (total)}} \times 100$$

The optical density units for all oligomers were multiplied by a small constant (1.04) to correct for hypochromism (Cantor et al., 1969).

Results

Figure 1 A shows the column chromatography of thymidine oligonucleotides, formed in a typical reaction, while Figure 1 B illustrates the effects of bubbling small amounts of HCl gas through an identical reaction. Chromatographic peaks are identified by comparing their R values, obtained in two solvent systems, with those given in the literature (Table 1). The increase in area of peak 1 (Fig. 1 B) which consists of thymidine, shows that the addition of a small amount of HCl gas greatly increases the hydrolysis of 5′-TMP. Although there is less total yield of oligomers in the presence of HCl, there is a greater

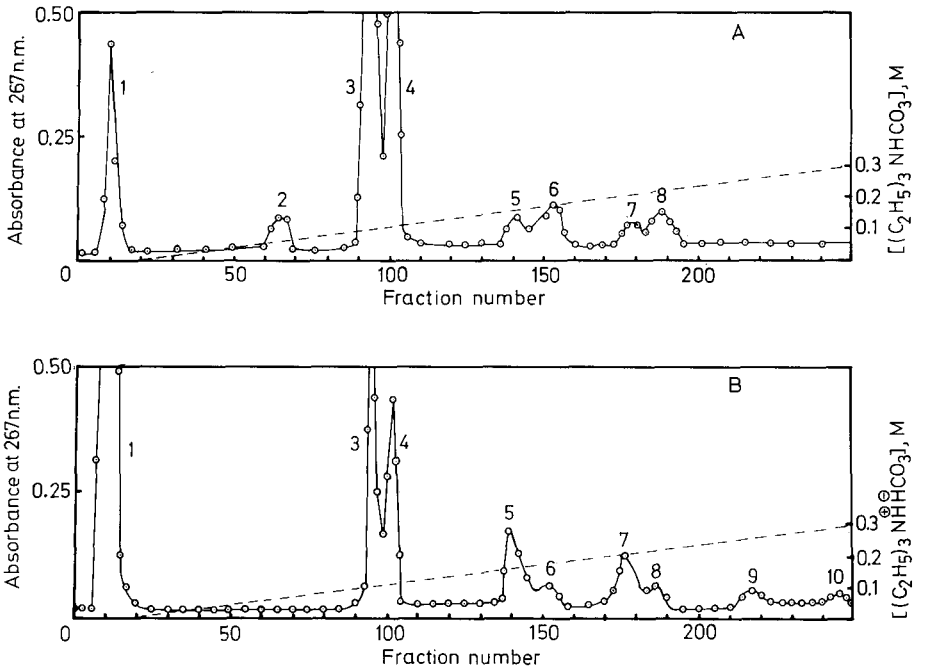

Fig. 1. Anion Exchange chromatography of thymidine oligonucleotides, on a DEAE cellulose column; using 0.30 M linear $(C_2H_5)_3NHCo_3$ gradient, formed from 5′-TMP in hexane: benzene: water:DACl:dicyanodiimide system in the absence (Fig. 1A) and in the presence (Fig. 1B) of HCl gas. Peaks are listed in Table 4

Table 1. Identification and R-values of chromatographic peaks[a]

Peak No.[b]	Solvent system[c]	R-value[a] This work	Literature[d]	Product
1	A			Thymidine
2	A	2.50	2.65	Thymidine 3′, 5′-cyclic phosphate
3	A	1.00	1.00	Thymidine 5′-phosphate
4	A	1.46	1.4	Cyclic dinucleotide
5	A	0.55	0.54	Linear dinucleotide
6	A	0.55	0.53	Cyclic trinucleotide
7	A	0.29	0.28	Linear trinucleotide
8	A	0.26	0.4	Cyclic tetranucleotide
9				Linear tetranucleotide
10				Pentanucleotide
2	B	1.5	1.3	
4	B	0.80	0.75	
5	B	0.70	0.68	
6	B	0.50	0.51	
7	B	0.49	0.49	
8	B	0.36	0.35	

a Relative to 5′ TMP
b In Figure 1A
c See Experimental Procedure
d Khorana and Vizsolyi, 1961

Table 2. Effects of surfactants on TMP oligomerization

	Surfactant[a]	% Total oligomers formed[b]
Cationic	Dodecylammonium chloride	26
	Hexadecyltrimethylammonium bromide	22
	Dodecylammonium propionate	3
Anionic	Sodium dodecanoate	0
	Sodium dodecyl sulfate	0
	Lauroyl histidine	< 2
	None	< 2

a In 10 ml benzene + 10 ml hexane, [Surfactant] = 4.5×10^{-3} M [TMP] = 3.0×10^{-3}. At 72.0° subsequent to 7 day reaction, H_2O = 0.01 M

b For % yield determination, see Experimental Procedure

percentage of linear and higher molecular weight oligomers. Apparently the hydrolysis products of cyclic oligomers react with 5′-TMP resulting in an enhanced yield of the larger oligonucleotides. In addition to paper chromatographic identification, the oligonucleotides in peaks 5 and 7 were subjected to enzyme degradation (see Experimental Procedures). Ratios of absorbance of TMP to thymidine were found to be 0.95 and 1.90 for peaks 5 and 7, respectively. These observations substantiate that peaks 5 and 7 correspond to the linear dimer and trimer, respectively.

Oligomerization of 5′-TMP in apolar solvents can be catalyzed, inhibited, or not affected by surfactant aggregates. This is shown in Table 2.

In general, cationic surfactants catalyze nucleotide oligomerization and anionic surfactants inhibit it.

The oligomerization of 5′-TMP in apolar solvents is very temperature dependent. This is shown in Figure 2. At 21 °C there is about 2–3 % product and it is nearly all linear dimer, whereas at 72 °C there is 26 % product and it is nearly all cyclic oligomers (unless a small amount of HCl gas is added).

The variations in concentrations of 5′-TMP, thymidine, 3′,5′-cyclic TMP, dimer, trimer and tetramer with time is shown in Figure 3. As one might expect, the concentration of oligomers and the hydrolysis product, thymidine, continually increase with time until the TMP becomes depleted.

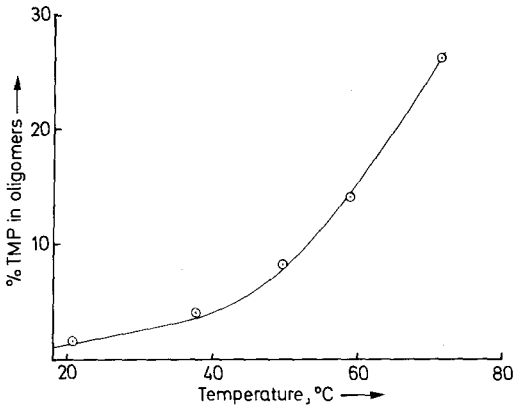

Fig. 2. Temperature dependence of TMP oligomerization. All experiments have been carried out for 7 days. See Experimental Procedure

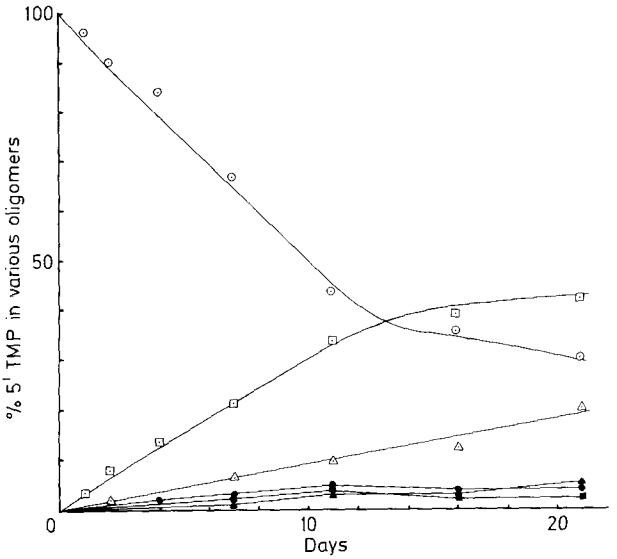

Fig. 3. Variation of 5′-TMP (○), thymidine (△), 3,5-cyclic-TMP (▲), total dimers (□), total trimers (●) and total tetramers (■) with time in the oligomerization of 5′-TMP at 72°

Several modifications of the basic oligomerization reaction (described in the Experimental Procedure) were tried in an attempt to optimize our yield and also to determine the practical limits of DACl as a catalyst (see Table 3). In separate experiments, 3′,5′-cyclic TMP (10 mg) and magnesium chloride were added to the oligomerization reaction. If 3′,5′-cyclic TMP were an activated intermediate in the oligomerization reaction, its addition at the beginning of the reaction would result in a greater yield in a shorter time. What we found was that addition of cyclic TMP (in amounts up to 50 % of the total TMP) had no affect on oligomer formation. It is well known the Mg^{2+} forms strong complexes with polynucleotides (Izatt et al., 1971). It was hoped that addition of $MgCl_2$ to the reaction would result in an enhanced yield. Mg^{2+} was also chosen because it is the second most prevalent cation in sea water (after Na^+). Unfortunately, no additional catalysis was observed. In order to demonstrate the ability of DACl to cata-

Table 3. Oligomer yields in different reactions[a]

Variation from reaction A	% Oligomerization[b]	% Hydrolysis
1 day	3.5	< 1.0
2 days	8.0	2.0
4 days	16.5	3.0
7 days	26.0	6.5
11 days	40.0	10.5
16 days	44.0	12.0
21 days	50.0	20.0
10 mg 5′-TMP+10 mg 3′,5′cyclic TMP	12.0	4.0
$2x10^{-7}$ moles 5′-TMP ($1x10^{-5}$ M)	27.0	
432 mg DCC (no dicyanodiimide)	< 1.0	
10 mg $MgCl_2$	24.0	
$6x10^{-5}$ moles thymidine + $1.2x10^{-4}$ moles K_2HPO_4 (no 5′-TMP)	% 5 TMP formation	

a The standard reaction, A, = 20 mg 5′-TMP, 200 mg DACl and 200 mg dicyanodiimide stirred for 7 days at 72° C in 10 ml hexane + 10 ml benzene. H_20 = 0.01 M

b For % yield determination, see Experimental Procedure

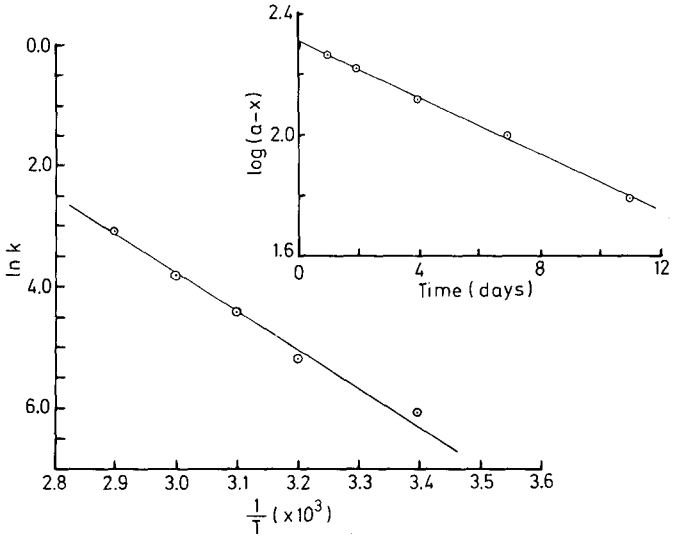

Fig. 4. Arrhenius plot for the oligomerization of 5'-TMP. The insert shows a typical kinetic plot for oligomerization at 72°

lyze the oligomerization reaction in extremely dilute solutions, 1 x 10^{-5} M TMP was allowed to react under the usual conditions for 7 days. After concentration and chromatographing of the product, 27% of the cyclic dimer was found. DCC was found to be ineffective as a dehydrating agent in this system because of the presence of water.

DACl reversed micelles also have the ability to catalyze the phosphorylation of thymidine. 1 x 10^{-2} M thymidine and 2 x 10^{-2} M K_2HPO_4 were placed in a reaction vessel in the same conditions as the oligomerization occured (72 °C, 7 days). Yields between 2 and 5% TMP were formed. Both the 3' and the 5' isomers were formed in about equal amounts.

The formation of oligomers appears to be first order process (see Fig. 4, insert). Apparent first order rate constants are given in Table 4.

The energy of activation (*Ea*) of 5' TMP oligomerization was determined from the satisfactory Arrhenius plot (Fig. 4) of Equation 1.

$$ln\ k = ln\ A - \frac{Ea}{R}\left(\frac{1}{T}\right) \qquad (1)$$

Table 4. Rate constants for oligomerization [a,b]

Temperature °C	$10^{-3}k_{app}$, day^{-1}
21.0	2.36
38.0	5.74
50.0	12.0
59.0	21.6
72.0	46.6

a Using DACl as the surfactant
b Based on d[TMP]dt, excluding the TMP which was hydrolyzed

From the Ea, 12.4 ± 1.9 Kcals/mole, the enthalpy of activation ($\Delta H^{\ddagger}$) was determined to be 11.8 ± 1.9 Kcals/mole at 294 °K (Equ. 2).

$$\Delta H^{\ddagger} = Ea - RT \tag{2}$$

The entropy of activation ($\Delta S^{\ddagger}$) and the free energy of activation ($\Delta G^{\ddagger}$) were calculated from the standard Equations 3 and 4 respectively.

$$\frac{\Delta S^{\ddagger}}{2.303R} = \log k - \log \frac{ek}{h} - \log T + \frac{Ea}{2.303RT} \tag{3}$$

$$\Delta G^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger} \tag{4}$$

The $\Delta S^{\ddagger}$ was determined to be −53 ± 6 entropy units and $\Delta G^{\ddagger}$ was 27.4 ± 4.0 Kcals/mole (at 294 °K).

Discussion

The need of accommodation to prebiotic conditions renders the problem of nucleotide polymerization one of the most formidable challenges in prebiotic chemistry. Some typical experiments are summarized in Table 5 for easy comparison of yields and methods. It is notable that the condensation of ribonucleotides via transesterification is satisfactory, while no method at present is capable of producing template active oligodeoxyribonucleotides (i.e., chainlength of 8 or more).

The approach of micellar polymerization seems to offer some advantages. Reversed cationic micelles are able to concentrate nucleotides and their polymers in extremely dilute solution. This is the first effective alternative to the "evaporating basin" hypothesis for concentrating nucleotides (or other prebiotic moieties) sufficiently for a reaction to occur. We have not been able to eliminate the predominance of cyclic oligonucleotides which are kinetically favored over linear oligonucleotides. A prebiotic mechanism, to prevent cyclization or to partially hydrolyze cyclic oligonucleotides needs to be discovered to allow the formation of even larger oligonucleotides. Micelles with aromatic functionality (i.e., purine or pyrimidine rings) may well be an improvement in this regard, since they may act as templates thereby restricting the free motion of linear oligonucleotides. The use of templates has been previously proposed (Steinman and Cole, 1967) and shown to be useful in nucleotide oligomerization (See Table 5). It is interesting to note that Verlander et al. (1973) used alkylamines to catalyze their solid state oligomerization. Our cationic micelles are composed of various long chain length alkyl amines. This and the fact that alkyl amines bind strongly to polynucleotides indicate that alkylamines may have played a key role in the chemical evolution of polynucleotides.

Micelles thus far have been shown to selectively uptake amino acids, nucleosides, nucleotides, concentrate nucleotides from dilute solution and to catalyze their oligomerization (Nagyvary et al., 1976; Fendler et al., 1975). These are the requirements needed for a model system which explains the evolution of codon assignments.

Table 5. Nucleotide polymerization under prebiotic conditions

Starting Nucleotide	Medium	Temperature °C	Reaction time	Dehydrating agent	Catalyst or template	Linkage	Yield	Reference
5′-GMP and 5′-AMP, 0.01–0.025M	H_2O	~5–60°	2–17 days	CDI[a]	Polycytidilyc and polyuridylic acid	2′–5′	dimer = 0.1–6.9%	Sulston et al., 1968, 1969
Adenosine 5′-phosphorimidazolide, 0.0125–0.025M	H_2O	0–50	1–14 days	CDI[a]	Zn^{2+} and polyuradylic acid	2′–5′	dimer = 0.4–27.6% trimer = 0.1–5.0% tetramer = 0.2–1.2%	Weimann et al., 1968; Sawai and Orgel, 1975
Adenosine 2′, 3′-cyclic phosphate	Solid state	25–85	1–2 days	–	Alkyl amines	3′–5′> 2′–5′	dimer = 32.7% trimer = 13.1% tetramer = 7.0% pentamer = 5.0% hexamer = 3.5% larger = 5.0%	Verlander et al., 1973
Cytidine 2′, 3′-cyclic phosphate	Solid state	65	1 h	Polyphosphoric acid	–	3′–5′> 2′–5′	Average chain length = 5.6, 2.6%	Schwartz and Fox, 1967
5′-TMP, 0.125M	H_2O	70	1 day	–	Imidazole	3′–5′	Dimer < 0.2% trimer < 0.1%	Ibanez et al., 1971[a]
5′-TMP 0.125M	H_2O	90	1 day	Cyanamide	Montmorillinite	3′–5′	Dimer = 0.06–0.9% trimer = 0.01–1.6% tetramer = 0.003–0.05% cyclic tetramer = 0.02% cyclic pentamer = 0.05%	Ibanez et al., 1971b
5′-TMP. 1.0×10^{-5}M–3.0×10^{-3}M	hexane/benzene 0.01M H_2O	21–72	1–21 days	Dicyanodiimide	Surfactant aggregate	3′–5′	cyclic TMP = 3–5% cyclic dimer = 40% linear dimer = 1.1–9.0% cyclic trimer = 4% linear trimer = 1–5% cyclic tetramer = 1–4% larger < 1.5%	This work

[a] 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride

According to theories of molecular self-organization (Eigen, 1971; Kuhn, 1972) the transition from molecular chaos to a highly ordered living system is very unfavorable entropically. Hence, the thermodynamics of reactions involved in chemical evolution should be of considerable interest. Our experimental model provided a unique opportunity to calculate the activation energy of a 5′-TMP oligomerization reaction. The value of $\Delta G^{\ddagger} = 27.4 \pm 4.0$ Kcals/mole is in the region of the observed for numerous other reactions (Oshima and Iwai, 1968). The value of $\Delta S^{\ddagger} = -53 \pm 6$ e.u., however, is large. It appears that oligonucleotide formation in our particular system is entropy controlled. Living systems overcome entropically unfavored reactions largely by the use of enzymes. Perhaps micelles or some other primitive enzyme model may function in a similar manner.

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