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Trivaline 'catalyzes' 5′-pdGTT oligomerization in solution

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We have found that the 5′-pdGTT molecules at a concentration of 10^{-4} M are oligomerized in solution in the presence of 10^{-4} M tripeptide – (*L*-Val)$_3$-NH-NH-DNS·CF_3COOH and the condensation reagents (carbodiimide and imidazole). Oligonucleotides not less than 12 bases long were formed in the yield which was over 15%. It is known that in the absence of peptide 10^{-2} M mono- or dinucleotides are required. Thus trivaline can be considered as one of the simplest enzymes. This oligomerization seems to be an essential way for the synthesis of long enough oligonucleotides of the random GC-sequence, which could be used at the earliest steps of evolution.

Trinucleotide; Tripeptide; Oligomerization; Evolution

1. INTRODUCTION

It has been established by now that in the presence of condensing agents at least 10^{-2} M concentration of mononucleotides is necessary for polymerization without polynucleotide template and at least 10^{-3} M if such a template is present in solution [1,2]. The template concentration expressed in that of phosphate is 10^{-2} M. Even if a mononucleotide is replaced by the dinucleotide – 3′-pdAppAp-5′ – its concentration cannot be lower than 10^{-2} M [3]. Although the presence of polynucleotide templates in the 'initial clear soup' is hardly possible, the presence of short peptides in it is very likely [4]. We assume that evolution of peptides and nucleic acids took place hand in hand from their earliest steps. It is known that short peptides can bind to nucleic acids (see review [5]).

During the past 15 years we have studied trivaline interaction with double-stranded [5–7] and single-stranded [8] nucleic acids. Some of those peptide–nucleic acid complexes were characterized by increased fluorescence and/or compaction capability. pdGTT were also able to form a highly fluorescent complex with trivaline. We supposed that the position of oligonucleotides in such complexes may allow 3′- and 5′-ends of adjacent oligonucleotide molecules to be drawn together and this in turn, may favor the formation of a phosphodiester bond between them.

The aim of this work was investigation of the 5′-pdGTT polymerization reaction under conditions favourable for the formation of a trinucleotide trivaline light fluorescent complex.

2. MATERIALS AND METHODS

2.1. *Samples and preparation of the nucleo-peptide complex*

5′-pdGTT was synthesized by the triester method in solution. DHTV was prepared as described previously [5]. The solution, containing double peptide concentration in the following buffer (1 mM sodium cacodylate, pH 6.6, 50% MeOH), was prepared as described elsewhere [5]. Another solution contained double 5′-pdGTT concentration in the same buffer without MeOH. To this solution EDAC and imidazole were added [9]. Both solutions were kept for 2 h at 4°C and then equal volumes of peptide- and nucleotide-containing solutions were combined. The reaction mixture was kept for 2–3 weeks at 4°C. It contained 1×10^{-4} to 5×10^{-4} M 5′-pdGTT, 2.5×10^{-4} to 12×10^{-4} M DHTV, 0.05 M of each EDAC and imidazole, 25% MeOH in 1 mM sodium cacodylate, pH 6.6. After 24 h the fluorescence intensities of the reaction mixture and free peptide at the same concentration were measured at λ_{ex} = 400 nm and λ_{em} = 500 nm.

2.2. *High pressure liquid chromatography (HPLC)*

HPLC was carried out in Zorbax ODS column with the particles 3 μm and the flow rate 1.2 ml/min. Solution A = 0.1 M ammonium acetate; solution B = 50% acetonitrile in 0.1 M ammonium acetate. The linear CH_3CN gradients 10–30% or 10–40% elution time 20–30 min were used and optical density was measured at 260 or 330 nm.

2.3. *Desalting of samples*

After HPLC fractionation of the reaction mixture oligonucleotides of each fraction were precipitated with 2% $LiClO_4$ in acetone followed by centrifugation and precipitate washing with acetone.

2.4. *Polyacrylamide gel electrophoresis (PAGE)*

Oligonucleotides of each fraction were labelled at the 5′-end with radioactive phosphate by the exchange reaction carried out by T4 polynucleotide kinase in the presence of [γ-^{32}P]ATP as described elsewhere [10]. Radioactively labelled oligonucleotides obtained in this

Abbreviations: EDAC, 1-ethyl-3-(3-dimethylaminopropyl)-3-carbodiimide hydrochloride; DHTV, (*L*-Val)$_3$-N_2H_2-DNS·CF_3COOH; DNS, 1-dimethylamino naphthalene-5-sulphonic acid; HPLC, high pressure liquid chromatography; PAAG, polyacrylamide gel; PAGE, polyacrylamide gel electrophoresis.

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way were fractionated by PAGE (20% PAAG, 0.4 mm thick, 20 × 30 cm) [11]. A radioactively labelled 14 base long oligonucleotide, partly hydrolyzed by the *E. coli* DNA polymerase I (Klenow's fragment), was used as a marker for length determination of obtained oligonucleotides.

3. RESULTS AND DISCUSSION

The intensity of the complex fluorescence, measured in 24 h after the reaction's beginning, turned out to be by 3–8 times higher than that of a free peptide in the same concentration. This fact indicates the formation of a highly fluorescent complex of DHTV with 5'-pdGTT in the presence of EDAC and imidazole [5–8]. Fig. 1 shows the elution patterns of the reaction mixture, original 5'-pdGTT and the peptide-lacking reaction mixture from the HPLC column. Comparison of the profiles in Fig. 1A and B shows that the reaction mixture contains oligonucleotides longer than the original trinucleotide. From the profiles in Fig. 1A and C follows that longer oligonucleotides arise in such condi-

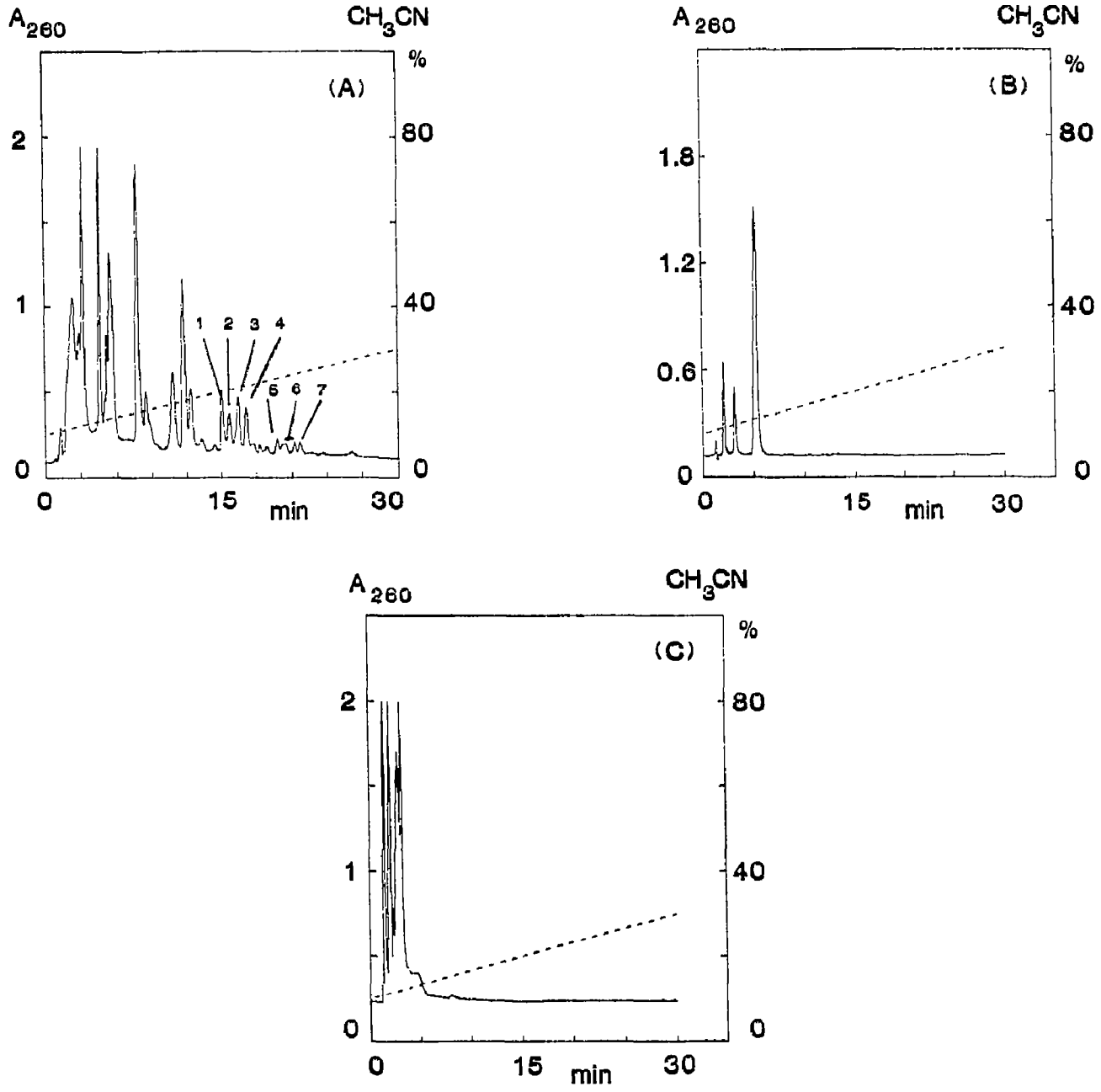

Fig. 1. Elution patterns of the reaction mixture during 3 weeks from the beginning of the reaction (A), original pdGTT (B) and reaction mixture without DHTV during 3 weeks from the reaction's beginning (C). Dotted line represents the gradient of CH_3CN concentrations in 0.1 M ammonium acetate. The reaction mixture contained: 1.3×10^{-4} M pdGTT, 10^{-4} M DHTV, 0.05 M EDAC, 0.05 M imidazole, 25% MeOH, 1 mM Na-cacodylate, pH 6.6.

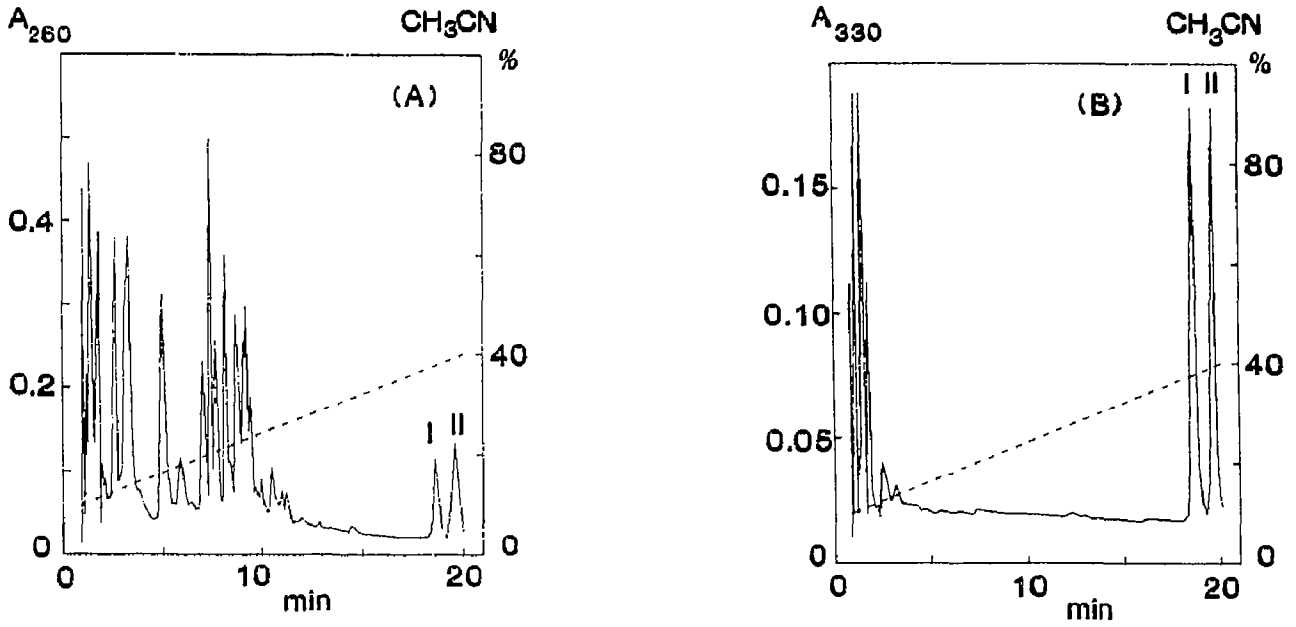

Fig. 2. Elution patterns of the reaction mixture during 3 weeks from the beginning of the reaction registered at 260 nm (A) and 330 (B). Dotted line represents the gradient of CH_3CN concentration in 0.1 M ammonium acetate. The reaction mixture is the same as in Fig. 1.

tions only if the peptide was present in the reaction mixture.

Although the dansyl group is also able to absorb at 260 nm, the optical density peaks in Fig. 1 belong to the nucleic acid only. Let us analyze Fig. 2A and B. They show the elution patterns of the same reaction mixture registered at 260 nm (A) and 330 nm (B).

Comparison of elution patterns in Fig. 2A and B shows that at a CH_3CN concentration lower than 35% the optical density peak corresponds only to the nucleic acids.

Besides peaks in Fig. 2A, qualitatively identical to those in Fig. 1A, there are two other peaks in Fig. 2A – I and II at CH_3CN concentration over 35%. Their position in Fig. 2A and B is the same. As was shown by the experiment with the reaction mixture containing the peptide alone (without 5′-pdGTT), peak I corresponds to the free peptide, while peak II, evidently, corresponds to the peptide covalently linked with 5′-pdGTT, rather than by a N–P link. Really, although optical densities at 330 nm were the same in both peaks, the absorption of peak II at 260 nm is higher, probably due to its nucleic bases.

For estimation of the oligonucleotide lengths from peaks 1–7 (Fig. 1A) they were analyzed by PAGE. The electrophoregram is given in Fig. 3. The following facts have drawn our attention: (i) that oligonucleotides at least 6 and 12 bases long are present in the solution; (ii) the fact that molecules consisting of 12 bases are eluted from the column at different rates.

Their different chromatographic mobility may be caused both by the presence or absence in them of the end phosphate and a possible formation of the 5′–5′ phosphodiester or pyrophosphate bonds during oligomerization. Compounds of this type, arising in this reaction mixture were described elsewhere [12].

Our investigations allow us to conclude that under the conditions used here trivaline plays a role of an unspecific template or 'catalyst' favourable for poly-

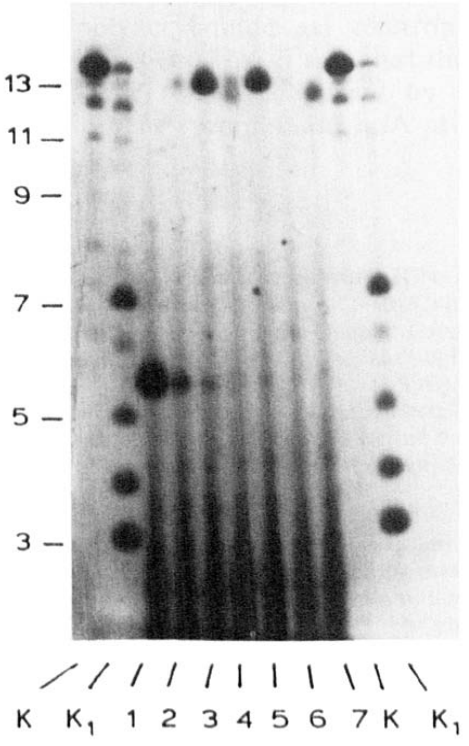

Fig. 3. Electrophoresis in PAAG. Vertical axis is marked by oligonucleotide lengths from 3 to 14; K = a polynucleotide 14 bases long; K_1 = the same polynucleotide partly hydrolyzed in advance; 1–7 = fraction numbers, corresponding to those in Fig. 1A.

merization of trinucleotides at their abnormally low concentration (10^{-4} M). We have shown earlier [8] the low specificity of the highly fluorescent trivaline complex formation, concerning the nucleotide sequence. It has been shown that other peptides of similar composition are also able to form highly fluorescent and compact complexes with nucleic acids [5].

Thus, the short peptides able to form β-dimers in solution [5], may 'catalyze' the trinucleotide polymerization reaction at concentrations a hundred times lower than has been known up until now.

We think that trinucleotides of an arbitrary composition and original tripeptides can also be formed by an abiotic way [1–4]. A qualitatively new stage of evolution had begun when the trinucleotides and some tripeptides appeared in solution. We have shown in this paper that trivaline catalyzes the formation of a 12-mer from trinucleotides. But also the 12-mer and more extended oligonucleotides can form the same complexes as trinucleotides with tripeptides. So, step by step the extended-enough oligonucleotides can be formed. The base sequences of oligonucleotides thus obtained must be random, because highly fluorescent trivaline complexes have no specificity to any nucleic bases [5]. Among these oligonucleotides one can select the molecules which correspond to the biologically important oligopeptides, namely such oligopeptides that catalyze the oligonucleotide covariant reduplication and synthesis of oligopeptides themselves along the oligonucleotide templates according to the triplet code. And so the simplest self-reproducible biological system could arise.

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