SELECTIVE BINDING OF OLIGORIBONUCLEOTIDES BY *E. COLI* RNA POLYMERASE AND THEIR EFFECT ON DNA-DEPENDENT RNA SYNTHESIS

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1. Introduction

Recent studies from this laboratory have shown that *E. coli* RNA polymerase (EC 2.7.7.6) binds selectively certain oligoribonucleotides of a chain length $\geqslant$ 5 residues from random isoplith mixtures obtained by endonuclease digestion of RNA and by subsequent fractionation of the hydrolysate [1]. The purpose of this work was further investigation of the interaction of oligoribonucleotides and *E. coli* RNA polymerase, and the study of their effect on transcription. It was shown that some of the conditions for the binding of pentaribonucleotides by *E. coli* RNA polymerase are similar to those required for DNA transcription by the enzyme. Adding an excess of the enzyme to the random mixture of pentaribonucleotides exhausts all those oligomers which are capable of being bound. These pentanucleotides compete with RNA for *E. coli* RNA polymerase and inhibit transcription.

2. Materials and methods

E. coli RNA polymerase obtained according to the modified method of Babinet [2] (spec. act. 350-800 U/mg in Hurwitz units [3]) was purchased from the Special Bureau for Production of Biologically Active Substances (Novosibirsk, U.S.S.R.). For some experiments RNA polymerase has been separated into core enzyme and σ-subunit by phosphocellulose column chromatography [4]. Isoplith fractions of oligoribonucleotides were prepared as described earlier [1] from enzymic hydrolysate of highly labelled RNA. The latter was obtained by transcription of calf thymus DNA using *E. coli* RNA polymerase and H^3-labelled ribonucleoside triphosphates or extracted from *E. coli* grown in the medium with P^{32}-orthophosphate. For RNA hydrolysis nonspecific endonuclease from cobra venom was used [5].

3. Results and discussion

3.1. *Conditions for binding*

As shown in fig.1, the optimal ionic strength for the binding of *E. coli* RNA polymerase to pentaribonucleotides is approx. 0.2. This value coincides with the optimal ionic strength for transcription [6]. The increase of ionic strength in both cases leads to the dissociation of the complexes. The binding of the pentaribonucleotides by the enzyme reaches a maximum very

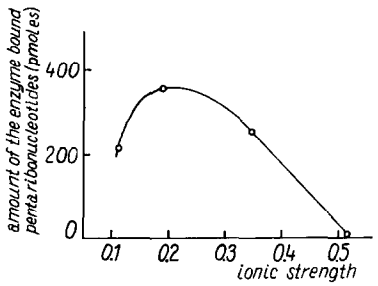

Fig.1. Effect of ionic strength on the binding of pentaribonucleotides with *E. coli* RNA polymerase. The enzyme (134 U/ml) was incubated for 30 min at room temperature with pentanucleotides (44.8 nmol/ml) in buffer containing 0.01 M Tris–HCl (pH 8.0), 0.01 M $MgCl_2$, 0.01 M β-mercaptoethanol, 0.001 M EDTA and NaCl from 0.05 M to 0.45 M.

rapidly (< 2 min), as in the case of DNA, and similarly occurs in the absence of Mg^{++} (or Mn^{++}) and ribonucleoside triphosphates.

Preincubation of RNA polymerase with 10^{-3} M *p*-chloromercurybenzoate (PCMB) sharply inhibits the capacity of the enzyme to bind pentaribonucleotides. The blocking of RNA polymerase SH-groups by PCMB hinders also the binding of the enzyme to DNA, initiation and elongation of RNA transcription [7]. In the presence of 6.3 M urea the formation of the complexes of *E. coli* RNA polymerase and pentaribonucleotides were also inhibited [8]. The results presented in table 1 demonstrate that separation of the σ-subunit from the enzyme does not diminish its binding capacity; the σ-subunit by itself does not bind pentaribonucleotides. It seems probable that the σ-subunit plays an important role only in the recognition of a specific site for the initiation of RNA synthesis on double-stranded DNA, facilitating the unwinding of this site [9]. The enzyme deprived of the σ-subunit preserves, obviously, the capacity to recognize specific sequences on a single-stranded molecule. The data obtained indicate that a number of conditions for the initiation of transcription and for the formation of the complexes between *E. coli* RNA polymerase and pentaribonucleotides are rather similar.

3.2. *Exhaustive binding of the selected pentaribonucleotides*

During incubation of *E. coli* RNA polymerase with a random set of pentaribonucleotides the enzyme binds a relatively small amount of them which differs depending on the depth of hydrolysis. As shown in table 2, the amount of the enzyme-bound pentanucleotides in experiment 1 was 0.35% and did not increase when the amount of the enzyme was doubled (experiment 2). When the enzyme-pentanucleotides complexes were separated by gel-filtration [1] and a new portion of RNA polymerase was added to the rest of the mixture, the amount of the enzyme-bound pentanucleotides fell to 0.05%. When the complex was separated and a third portion of the enzyme was added, the same small amount of pentanucleotides was bound (experiment 1). The enzyme binds the same small amounts of olygonucleotides when their length is < 5 due, probably, to unspecific adsorption (experiment 3).

It was shown earlier that a part of the pentanucleotides is bound tightly to *E. coli* RNA polymerase and the rest is attached loosely [1]. The tightly bound pentanucleotides were separated from the enzyme by heat denaturation of the latter and by subsequent gel-filtration [9], and reincubated with RNA polymerase. In this case the amount of the enzyme-bound pentanuc-

Table 1
Role of σ-subunit in the binding of pentaribonucleotides by *E. coli* RNA polymerase

Experiment No	Conditions	Amount of pentaribonucleotides (nmol)	Molar ratio (enzyme/pentaribonucleotides)	% bound pentaribonucleotides
1	Holoenzyme 0.76 nmol	128.0	0.6 : 100	0.34
2	Core enzyme 0.50 nmol	45.6	1.1 : 100	0.30
3	σ-subunit 1.55 nmol	45.6	3.3 : 100	0.00

E. coli RNA polymerase (holoenzyme), core enzyme or σ-subunit was incubated with pentaribonucleotides at room temperature for 30 min in buffer containing 0.01 M Tris–HCl (pH 7.9), 0.01 M β-mercaptoethanol, 0.01 M NaCl and 0.001 M EDTA. Complexes were separated from unbound oligonucleotides by gel-filtration [1].

Table 2
Exhaustive binding of oligoribonucleotides by *E. coli* RNA polymerase

Experiment No	Oligoribonucleotides	Molar ratio of the enzyme to oligoribonucleotides	1st binding		2nd binding	3rd binding
			Bound oligonucleotide (%)	Tightly bound oligonucleotide (%)	Bound oligonucleotide (%)	Bound oligonucleotide (%)
1	penta-	1 : 100	0.35	47	0.05	0.05
2	penta-	2 : 100	0.38	–	0.05	–
3	tetra-	0.6 : 100	0.06	–	–	–
4	penta- (tightly bound fraction)	100 : 100	16.9	86	–	–

Conditions of incubation and separation as in table 1. The tightly bound fraction of pentaribonucleotides was obtained after thermal denaturation of *E. coli* RNA polymerase, previously incubated with the whole fraction of pentaribonucleotides [9].

leotides reached approx. 17% (experiment 4) which is approx. 45 times more than the value in the experiments in which the pentanucleotides were selected by the enzyme from the random mixture (compare experiments 2 and 4). Most of these enzyme-bound pentanucleotides belong to the tightly bound fraction (approx. 86%). This great increase of the binding happens in spite of the fact that the amount of the pentaribonucleotides in experiment 4 is 50 times less than in experiment 2.

3.3. *Competition between oligoribonucleotides and DNA for the enzyme. Inhibition of transcription*

As shown in fig.2, the addition of DNA to the incubation medium diminishes the amount of previously formed complexes of labelled penta- or hexanucleotides with *E. coli* RNA polymerase. Probably, oligoribonucleotides have a lower affinity for the enzyme than has DNA, as the former are almost completely displaced from the complexes when 1000 pairs of deoxyribonucleotides are added per molecule of oligomer (fig.2). Addition of all four ribonucleoside triphosphates (ATP, GTP, CTP and UTP - 0.4 mM of each) does not prevent the formation of the enzyme-oligoribonucleotides complexes. It means, obviously, that oligoribonucleotides are attached not to the substrate site of *E. coli* RNA polymerase but to the site responsible for the binding of DNA. Confirmation of this view comes from the experiments showing that penta- or hexaribonucleotides inhibit DNA-dependent RNA synthesis by *E. coli* RNA polymerase, while tri- and tetraribonucleotides which are not bound by the enzyme do not affect the transcription (fig.3). It is known that when the concentration of ATP, GTP, CTP and UTP in the incubation medium is substantially lowered, the initiation of transcription becomes a limiting step [10]. When the concentration of ribonucleoside triphosphates in our experiments was diminished from 400 μM to 5 μM (fig.4) transcription was almost completely inhibited by the pentanucleotides and

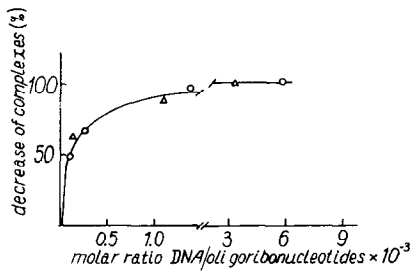

Fig.2. Effect of addition of DNA on the formation of complexes of *E. coli* RNA polymerase with oligoribonucleotides. Incubation conditions as described in table 1. Final concentrations of RNA polymerase: 65 U/ml, pentaribonucleotides: 25.6 nmol/ml (–○–○–) and hexaribonucleotides: 40.4 nmol/ml (–△–△–). The concentration of DNA was changed from 0 to 1.35 μmol of deoxynucleotides pairs per ml. The amount of degraded complexes was estimated as a difference between the amount of complexes formed without DNA and after addition of it.

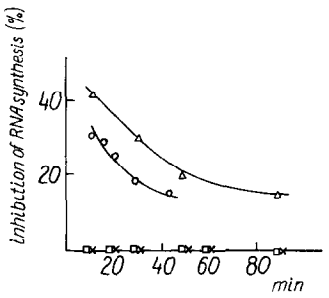

Fig.3. Effect of oligoribonucleotides of different length on DNA-dependent RNA synthesis by *E. coli* RNA polymerase. Incubation mixture (0.5 ml) contained 0.08 M Tris–HCl (pH 7.9), 10 mM β-mercaptoethanol, 8 mM $MgCl_2$, 2 mM $MnCl_2$, [^{14}C] ATP (specific activity 0.15 μCi/μmol), UTP, CTP and GTP 400 μM each, 50 U/ml of *E. coli* RNA polymerase, 280 μg/ml of DNA and oligoribonucleotides: tri- 40.4 nmol/ml (–X–X–), tetra- 40.4 nmol/ml (–□–□–), penta- 20.2 nmol/ml (–○–○–), hexa- 40.4 nmol/ml (–△–△–). RNA polymerase was preincubated with oligoribonucleotides 30 min at room temperature, then DNA was added and RNA synthesis was carried out at 37°C. The reaction was stopped by adding 2.0 ml of cold 5% trichloroacetic acid and 0.1 ml of 1% RNA solution. Precipitates were collected on AUFS millipore filters and the radioactivity was determined.

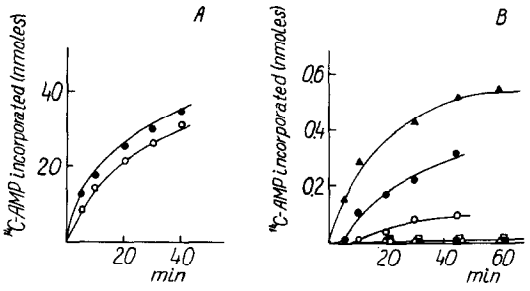

Fig.4. Effect of tightly and loosely bound pentaribonucleotides on DNA-dependent RNA synthesis by *E. coli* RNA polymerase. Incubation conditions as described in fig.3. In experiment A the specific activity of [^{14}C] ATP was 0.15 μCi/μmol and the concentrations of [^{14}C] ATP, UTP, CTP and GTP was 400 μM each, RNA polymerase: 32 U/ml and DNA: 232 μg/ml; (–●–●)control, (–○–○–) pentaribonucleotides, 40 nmol/ml. In experiment B the specific activity of [^{14}C] ATP was 100 μCi/μmol and the concentrations of [^{14}C] ATP, UTP, CTP and GTP were 5 μM each, RNA polymerase: 36 U/ml and DNA: 100 μg/ml. (–●–●) control (–○–○) pentaribonucleotides, 42.0 nmol/ml; (–△–△) pentaribonucleotides, 85.0 nmol/ml; (–■–■–) tightly bound pentaribonucleotides, 0.4 nmol/ml; (–□–□–) loosely bound pentaribonucleotides, 0.35 nmol/ml (–▲–▲–) pentaribonucleotides depribed of the RNA polymerase bound oligonucleotides.

especially by their loosely and tightly bound subfractions. The latter two were effective in concentrations 100–200 times lower than the whole fraction. The data are consistent with the assumption that oligoribonucleotides inhibit the initiation stage, mimicking the promoter DNA regions rather than other regulatory sites.

The mixture of pentaribonucleotides deprived of the enzyme-bound oligomers not only does not inhibit transcription but even stimulates it (fig.3). It seems probable that these pentanucleotides contain sequences which serve for the initiation of RNA chains. It is known, for instance, that complementary oligonucleotides stimulate RNA synthesis when homopolynucleotides are used as templates [12].

Preliminary data have shown that RNA polymerases obtained from micro-organisms other than *E. coli* recognize and bind different pentaribonucleotides. The data suggest the possibility of using specifically bound oligonucleotides for the selective inhibition of transcription.

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