SPECIFIC BINDING OF OLIGORIBONUCLEOTIDE FRACTIONS TO *E. coli* RNA POLYMERASE

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1. Introduction

Some data available concerning the specificity of interaction between RNA polymerase and polynucleotides [1, 2]. Such specificity was demonstrated by the difference in the affinity of RNA polymerase to various synthetic polynucleotides [1]. The purpose of this work was to clarify whether *E. coli* RNA polymerase is able to bind specifically the oligonucleotides of definite size and composition. Our findings indicate that certain oligoribonucleotide fractions from ribosomal RNA enzymatic digest bind preferentially to *E. coli* RNA polymerase.

2. Materials and methods

RNA polymerase from *E. coli* obtained according to the modified method of Babinet [3, 4] (specific activity 400–500 U/mg in Hurwitz units [5]) was purchased from the Special Bureau for Production of Biologically Active Substances (Novosibirsk, USSR). To prepare a random isoplite mixture of oligoribonucleotides ribosomal [^{32}P]RNA from *E. coli* (initial specific activity 7.5 mCi/mmole) was digested with ribonuclease from cobra venom and separated chromatographically according to Tomlinson and Tener [6]. The preparation of enzymatic hydrolysate of RNA and the estimation of nucleotide composition of oligoribonucleotides was performed as described in [7].

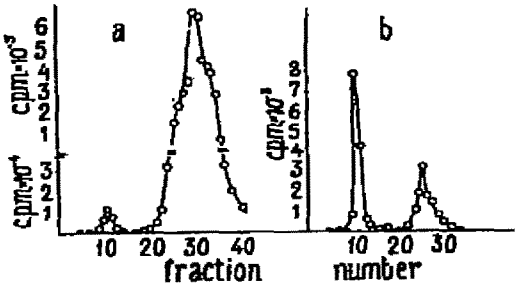

Fig. 1. Separation of the complex of RNA polymerase with pentaribonucleotides on Sephadex G-75. a) 1.74 mg of RNA polymerase were incubated for 10 min at 20° with 435 nmoles of [^{32}P]pentanucleotide mixture in 0.1 M Tris-HCl pH 7.9 with 0.005 M β-mercaptoethanol in 2 ml, were applied to the Sephadex G-75 column (1 × 30 cm) and were eluted with the same buffer. Fraction volumes 1 ml. b) Refiltration of the pentanucleotide–RNA polymerase complex from the previous experiments on Sephadex G-75 column (1 × 50 cm). Fraction volumes 2 ml.

3. Results and discussion

The ability of RNA polymerase to bind oligonucleotides was studied by a method proposed by one of the authors [7]. The procedure includes incubation of the enzyme with a mixture of oligoribonucleotides of definite length and random composition obtained by digestion of RNA from *E.coli* with nonspecific endonuclease from cobra venom and subsequent chromatography of the digest in Tomlinson and Tener's system [6]. The enzyme–oligo-

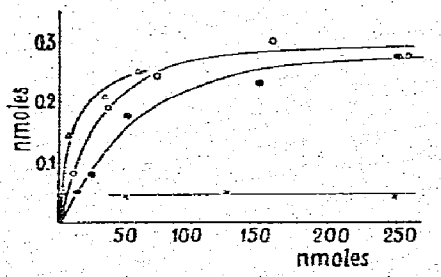

Fig. 2. The dependence of oligonucleotides binding to RNA-polymerase on the concentration of total oligonucleotide. 140 μg RNA polymerase were incubated with 0.5 ml oligonucleotide mixture under conditions described in fig. 1. Complex separated on Sephadex G-75 column (1 × 10 cm). Ordinate axis, nmoles of bound oligonucleotide. Abscissa axis, nmoles of total oligonucleotide. (×—×—×) = Tetranucleotides; (●—●—●) = pentanucleotides; (○—○—○) = hexanucleotides; (△—△—△) = heptanucleotides.

nucleotide complexes formed were separated nucleotide complexes formed were separated from free oligonucleotides by gel-filtration. The results of chromatography of the mixture of [^{32}P]pentanucleotides with RNA-polymerase are illustrated in fig. 1a.

Fig. 2 demonstrates the dependence of the degree of binding on the concentration of the tetra-, penta-, hexa- and heptaoligonucleotides incubated with the enzyme. For penta-, hexa- and heptanucleotides saturation takes place. At the initial part of the curve (enzyme excess) only 0.11% of the total amount of pentanucleotides, 0.28% of the hexa- and 0.44% of heptanucleotides bind to RNA polymerase. These data suggest specificity of binding. To prove this specificity the oligonucleotide mixture was incubated with excess of the enzyme, the complex formed was separated and the remaining oligonucleotide mixture was repeatedly incubated with new portions of enzyme. No binding occurred, thus proving that only a defined small part of the random oligonucleotide mixture binds to enzyme.

Rechromatography of the complexes results in a partial separation of labelled oligoribonucleotides attached to the enzyme (fig. 1b). While the loosely bound fraction dissociates in the course of the first gel-filtration the tightly bound oligonucleotides may be refiltered several times without appreciable loss of radioactivity from the enzyme fraction.

Table 1
Nucleotide composition of the pentanucleotides bound to RNA-polymerase.

Nucleotide	Composition (%)		
	Initial mixture	Loosely bound fraction	Tightly bound fraction
C	13.2	17	42.5
U	22.9	20.5	7.5
A	25.9	17.5	7.5
G	39	45	42.5

Nucleotide composition of both loosely and tightly bound fractions of pentanucleotides is presented in table 1. It may be seen that both fractions, especially the latter, are enriched in cytosine and guanine residues.

The nucleotide composition of the tightly bound fraction follows Chargaff rule (A = U; G = C), thus suggesting that complementary oligonucleotides interact with the enzyme. It is possible that RNA polymerase induces complementary oligonucleotide pairs of the random mixture to form short double helix fragments.

The data obtained show that RNA polymerase exhibits a preferential specificity towards several oligonucleotides with certain nucleotide composition, and that at least five nucleotide residues are necessary for measurable binding to the enzyme.

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