I_2 were similar, each having a positive band at 223 nm and a negative one at 204 nm.

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ISOLATION OF THE 5S rRNA of Phaseolus aureus. DETERMINATION OF THE STRUCTURE OF THE OLIGONUCLEOTIDES OF THE PYRIMIDYL-RNase FROM A HYDROLYSATE OF 5S rRNA

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The total rRNA from 12-hour shoots (30°C) of mung beans [1] was separated into two fractions by precipitation with 2 M NaCl in 0.25 M NaOAc buffer, pH 6. The high-molecular-weight rRNAs precipitated in this way were separated off by centrifugation and the concentration of NaCl in the supernatant was brought to 4 M, after which it was left at −10°C for 12 h. The resulting precipitate was removed, and low-molecular-weight RNAs were precipitated from the supernatant with ethanol. This salt-containing fraction was subjected to electrophoretic separation in 10% polyacrylamide gel in 0.05 M Tris-borate buffer with pH 8.3, 0.001 M EDTA, and 7 M urea. The RNA zones were stained with Methylene Blue without incubation of the gels in 1 M CH_3COOH. The zone corresponding to the 5S rRNA was cut out of the gel, homogenized in the elution buffer, and eluted by the phenol method [2]. The eluate was diluted fivefold with 0.01 M Tris-HCl, pH 7, and 7 M urea and was deposited on a microcolumn (1 × 40 mm) of DEAE-cellulose, after which the microcolumn was washed with water, ethanol, and water again and stepwise elution was performed with 0, 0.2, 0.65, and 1 M LiCl in 0.01 M Tris-HCl with pH 7, 7 M urea. The 5S rRNA was precipitated with three volumes of ethanol from the eluate after it had been concentrated in vacuum. The nucleotide composition of the mung bean 5S rRNA was determined by alkaline hydrolysis with 0.3 N

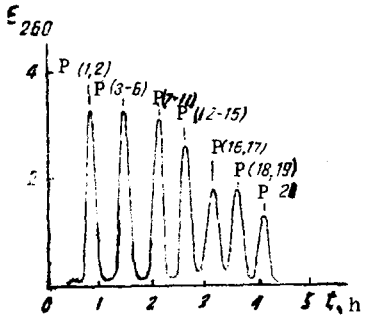

Fig. 1. MCLC separation of a pyrimidyl-RNase hydrolysate of mung bean 5S rRNA into isopleths.

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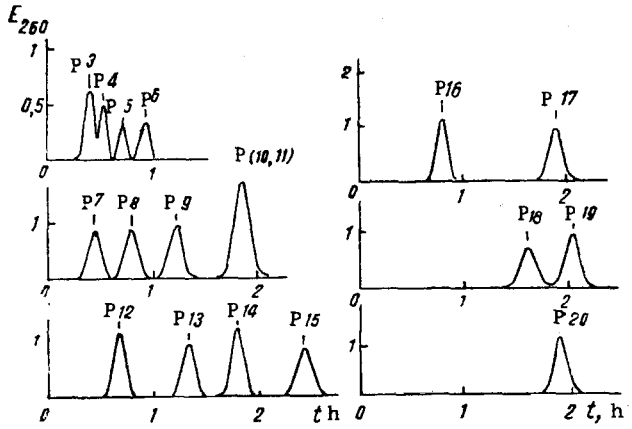

Fig. 2. Rechromatography of the isopleths at pH 3.7 (HCOOH) on a microcolumn (1 × 45 mm) containing DEAE-cellulose in the presence of 7 M urea with a concentration gradient of NaCl.

KOH at 37°C for 18 h. The K^+ ions were removed by passing the hydrolysate through a microcolumn (0.5 × 20 mm) of Dowex 50 × 2 cation-exchange resin. Then the hydrolysate was separated into nucleoside, mononucleotide, and nucleoside diphosphate fractions by the Tomlinson-Tener method [1]. The nucleoside fraction was identified by microcolumn liquid chromatography (MCLC) on a microcolumn (0.5 × 100 mm) containing Aminex-27 by a modification of the method described in [3]. The nucleotides were separated on a microcolumn (0.5 × 90 mm) with Dowex 1 × 8 [4]. The nucleoside diphosphate fraction was desalted [1] and was dephosphorylated with phosphomonoesterase (PME). The nucleoside obtained was identified by MCLC on Aminex-27. In this way the nucleotide compositions of the 3'- and 5'-ends of the mung bean 5S rRNA were determined:

AMP	GMP	CMP	UMP	3'-end	5'-end	G+C / A+U
22.4	28.8	25.9	22.9	U_{OH}	pAp	1.21

Pyrimidyl-RNase hydrolysis was performed with pancreatic RNase (Reanal) purified as described by Aqvist and Anfinsen [5], in 20 μl of 0.02 M Tris-HCl, pH 7.5, at 37°C with a ratio of 1 OU_{206} of 5S rRNA to 2 μg of enzyme for 12 h. The hydrolysate was diluted with a tenfold volume of 0.02 M Tris-HCl, pH 7.5, 7M urea and was separated into isopleths by a method described previously [1] (Fig. 1). The isopleths were rechromatographed by methods described previously [1, 6] (Fig. 2). The structures of the oligonucleotides were determined from the experimental results of:

a) determination of the 5'-end and the nucleotide composition;

b) hydrolysis with guanyl-RNase; and

c) partial hydrolysis with snake venom phosphodiesterase (PDE) of the oligonucleotides under investigation.

In the analysis under a) we used PME from calf intestine purified as described in [7] for the dephosphorylation of the oligonucleotides, and snake venom PDE for the complete hydrolysis of the oligonucleotides [6]. The hydrolysis products from a)-c) were analyzed by MCLC on a KhZh 1305 chromatograph [1, 4].

In this way the structures of all the oligonucleotides in the pyrimidyl-RNase hydrolysate of mung bean 5S rRNA were established (see Figs. 1 and 2; molar ratios were determined from the ratios of the amounts of 5'-terminal oligonucleotide): P1 – 15.7 Cp; P2 – 11.2 Up; P3 – 4.8 ACp; P4 – 5.1 GCp; P5 – 2.2 AUp; P6 – 2.9 GUp; P7 – 0.9 GACp; P8 – 1.1 AGCp; p9 – 1.2 AAUp; P10 – 2.1 AGUp (a); P11 – 0.9 GAUp (a); P12 – 1.0 AAGCp (a and b); P13 – 0.8 GGGCp (a); P14 – 1.1 GGAUp (a and b); P15 – 1.1 GGGUp (a), P16 – 0.7 AGAAp (a and b); P17 – 0.8 AGGAUp (a and b); P18 – 1.0 pAGGUp (a); P19 – 0.9 GAGAGUp (a and b); P20 – 0.8 GGGAAGUp (a and c).

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HYDROPHILIC POLYMERS FOR THE REVERSIBLE IMMOBILIZATION OF OLIGONUCLEOTIDES

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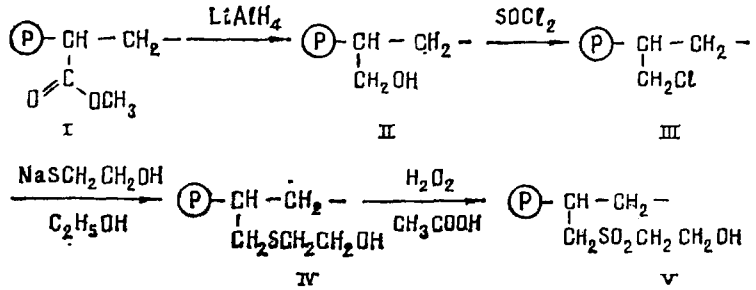
The aim of the present work was to create a hydrophilic polymer suitable for the reversible immobilization of oligonucleotides and for the development of methods for attaching oligonucleotides to a solid phase.

The polymeric matrices developed in our laboratory that have well recommended themselves in the solid-phase synthesis of oligonucleotides in both the diester [1] and the triester variants [2] consist of chemically modified polstyrene grafted onto the surface of an inert polytetrafluoroethylene (Teflon) matrix [3]. By the radiation grafting of monomers onto Teflon we have obtained a series of polymers possessing a hydrophilic nature of the grafted-on chains. This not only facilitates the migration of the oligonucleotide to the polymer but also lowers the nonspecific sorption of nucleotide material, which is usually due to hydrophobic interactions between the polymeric support and fragments of nucleic acids. The radiation grafting of the monomers was performed by a procedure published previously [2]. From this series, including ten different samples, on the basis of a number of criteria – hydrophilicity (wettability) of the polymer, which depends on the loading of the Teflon with monomers, nonspecific sorption, absence of ioonogenic groups, and reversibility of immobilization – we selected a polymer with a β-hydroxyethyl sulfide anchoring group.. In the construction of the anchoring groups of the polymeric supports, it was borne in mind that the immobilization of the oligonucleotides should be performed through the terminal phosphate groups of their molecules. This polymer was obtained by the scheme presented below:



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