ANALOGS OF PYRIMIDINE MONO- AND POLYNUCLEOTIDES. IV.* REACTION OF AN OLIGOTHYMIDYLIC ACID ANALOG BASED ON N-(1,4-DIHYDROXY-2-BUTYL)THYMINE WITH POLYADENYLIC ACID

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An oligothymidylic acid analog was obtained by polycondensation of N_1-(1,4-dihydroxy-2-butyl)thymine with its 1',4'-diphosphate in the presence of mesitylenesulfonyl chloride. The highest-molecular-weight fraction of the analog, which was isolated by gel filtration on Sephadex G-75, forms complexes with polyadenylic acid.

Our preceding communication [1] was devoted to the creation of a polythymidylic acid (PT) analog by polycondensation of N_1-(1,4-dihydroxy-2-butyl)thymidine (I) with N_1-(1,4-dihydroxy-2-butyl)thymine diphosphate (II) and polycondensation of the corresponding monophosphates (III). Low-molecular-weight oligomers with chains containing primarily diphosphate bonds were obtained when the latter polycondensation was carried out by the action of dicyclohexylcarbodiimide and triisopropylbenzenesulfonyl chloride. In contrast to this, polycondensation of equimolecular amounts of I with II under the same conditions gives oligomers containing a considerable number of pyrophosphate bonds.

The present communication is devoted to the synthesis and investigation of the properties of an oligothymidylic acid analog obtained by polycondensation of I and II with the aid of mesitylenesulfonyl chloride as the condensing agent at a nonequimolecular component ratio (I:II = 1.25:1).

Successive fractionation on Sephadex G-75 was used to isolate the relatively narrow high-molecular-weight fractions of the products from the reaction mixture. The molecular weights of the isolated oligomers were estimated by the method presented in [1].

The yield of the overall fraction of oligomers obtained by dialysis of the reaction mixture against water and the yields and distribution coefficients for the corresponding fractions obtained by the method indicated above are shown in Table 1.

It should be noted that whereas carrying out of the polycondensation with an equimolecular ratio of monomers I and II in the presence of mesitylenesulfonyl chloride does not lead to the formation of oligomers with sufficiently high degrees of polymerization, fraction No. 4, which has a distribution coefficient of 0.35, is formed in the case of a component molar ratio of 1.25:1 (see Table 1), and this distribution coefficient corresponds to an average degree of polymerization (n) of 27 with respect to the scale of oligothymidylates.

In addition, it is important to also point out that the oligomers contained in fraction No. 4 are only partially destroyed on treatment with 0.1 N HCl or acetic anhydride under the conditions of destruction of pyrophosphate bonds, and the nonhydrolyzed portion isolated by dialysis against water and 2 N NaCl and containing diphosphate bonds amounts to 70% of the

*See [1] for communication III.

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TABLE 1. Composition of the Products of Polycondensation of N_1-(1,4-Dihydroxy-2-butyl(thymine (I) with Its 1',4'-diphosphate (II)

Method of isolation	Yield, %	Distribution coefficient, K_d	Fraction No.
Dialysis against water	32,5	0,63	1
Gel filtration on Sephadex G-75	4,1	0,43	2
Repeated gel filtration on Sephadex G-75	0,8	0,40	3
Repeated gel filtration under the same conditions	0,24	0,35	4

reaction mixture. The average degree of polymerization of the oligomers isolated after hydrolysis is 10, according to the results of gel filtration on Sephadex G-25.

Thus the oligomers obtained at a component ratio of 1.25:1 correspond to a considerably greater degree to the desired objective than the polythymidylic acid analogs synthesized in the case of an equimolecular component ratio, which have lower molecular weights and a considerable number of pyrophosphate bonds.

It is known that definite information regarding the secondard structure of nucleic acids can be obtained by a study of the interaction of complementary oligo- and polynucleotides by determination of the hypochromic effect, which attests to stacking of purine and pyrimidine bases (for example, see [5-7]).

This method was also used for the study of the above-described polythymidylic acid analogs, in which the monosaccharide residues in the polymeric chain are replaced by the conformationally similar dibasic acid. We used the complementary polyadenylic acid (Poly A) as the second component for the study of the complexing. The results obtained in a study of the reaction of PT with Poly A in a citrate buffer are presented in Fig. 1. It was found that the oligomer with $K_d = 0.4$ is not hybridized with Poly A, but the higher-molecular-weight oligomer with $K_d = 0.35$ actually forms complexes with Poly A. Moreover, an interesting phenomenon — the formation of two components with different compositions, the first with 60% PT and 40% Poly A, and the second with 20% PT and 80% Poly A — is observed. Lowering the temperature and raising the ionic strength of the solution promote the primary formation of a 4Poly A:1PT complex, whereas raising the temperature and lowering the ionic strength of the solution to a great extent favor the formation of a 1.5PT:1Poly A complex.

It should be noted that the formation of two complexes with different compositions was also observed in [6, 7]. The peculiarity of the results that we obtained consists not in the formation of two complexes but rather in the existence between them of a sharp transition; this was not noted during an investigation of synthetic homopolynucleotides.

The overall stoichiometry of such complexes does not at all necessarily reflect the real stoichiometry of the contacting bases, inasmuch as complete interaction of the bases cannot occur between both forms of polymers in view of their structural differences. It is apparent that in our case, just as in the studies cited above, the first complex has a higher activation energy than the second.

The PT:Poly A complex melts at ~5-6°, although completion of the formation of the complex is not observed at —3° (Fig. 2); the hexathymidylate complex with Poly A has the same melting point [6].

Polythymidylic acid apparently has a tangled structure, inasmuch as the optical density of solutions of it does not depend on the temperature. However, one cannot exclude the possibility of random interactions between the thymine bases. In fact, we observed that the optical density of solutions of PT increases by 2% after heating at 100° for 1 h at pH 2, by 10% at pH 4, and by 7% at pH 13 as compared with the optical density of freshly prepared solutions of PT at the same pH values and room temperature. The minimal hypochromic effect, which is observed at pH 1, provides evidence that random interactions between the thymine bases are suppressed to a considerable degree right up to its destruction.

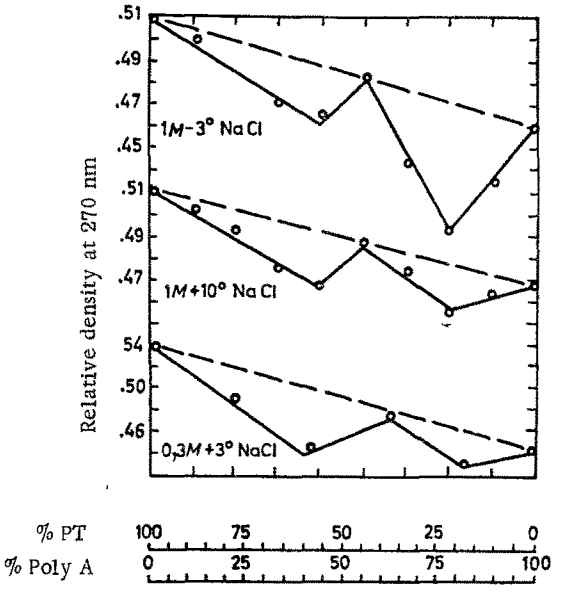

Fig. 1

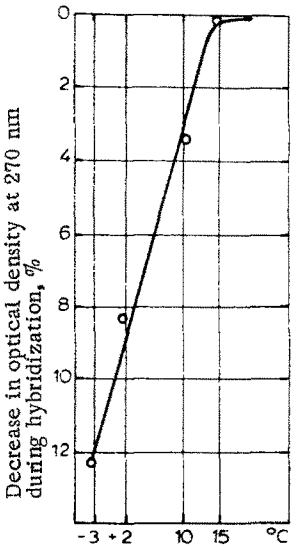

Fig. 2

Fig. 1. Result of experiments on the hybridization of Poly A and PT for K_0 = 0.35, pH 7.8, and a 0.015 M sodium citrate buffer.

Fig. 2. Temperature dependence of the decrease in the optical density of the 4Poly A:1PT complex in the presence of 0.015 M sodium citrate and 1 M sodium chloride at pH 7.8.

Considering the complexing of analogs of polynucleotides of the PT type with synthetic homopolynucleotides and the temperature stability of the associates formed in this case, one may note the fundamental qualitative peculiarities of these particles that determine the possibility of this sort of association: conformational similarity, chain length, polydispersed character, the presence of complementary bases, and the regular positioning of the bases along the chains of the polymers. In view of the asymmetrical orientation of the thymine base in monomers I and II and the presence of two different bonds between the monomeric links, the PT obtained is chemically irregular. In this case it is natural to expect that the macromolecular complex between Poly A and a similar partner will have a considerable number of "eyelets" and a considerably lower melting point than the complex formed by the same components and with the same chain length but regularly constructed. Inasmuch as the melting point and the width of the melting point interval characterize, as is well known, the cooperativeness of the interaction, considering the ideas expressed above, one should consider the low melting point of the investigated complex to be completely in conformity with principle.

In conclusion, it might be noted that the ability of the synthesized polythymidylic acid analogs to undergo hybridization with complementary polyadenylic acid that we established in this research provides a basis for the assumption that these oligomers may also have a definite biological effect on the biosynthesis of proteins and on replication of nucleic acids in the cell.

The results of a study of these phenomena will be reported in the next paper of this series.

EXPERIMENTAL

The preparation of absolute pyridine was described in [1]. Analytical grade mesitylenesulfonyl chloride was recrystallized from pentane and had mp 56°. The triethylamine was distilled twice and had bp 89.5°. The acetic anhydride was distilled twice.

The preparation of "fine" Sephadex G-75 did not differ from the method described in [1]. Dowex resin, treated by the method in [1], was washed in the column with two volumes of distilled water prior to use.

The potassium salt of Poly A (Reanal) contained a considerable amount of oligonucleotide impurities, which were removed by gel filtration on Sephadex G-75 in a 1N ammonium carbonate buffer. The outer and inner volumes of the column containing the Sephadex were determined from the elution volume of high-molecular-weight polyuridylic acid (molecular weight 6330) and sodium chloride. The distribution coefficient was determined as demonstrated in [1]. Chromatography was monitored by the method used in [1].

In the experiment for the investigation of hybridization, the cuvettes were placed in a thermostated cell connected to a cryostat cooled by ethanol–dry ice. The temperature in the cell was determined with an accuracy of ±1°.

Method Used for the Polycondensation. The polycondensation of 1.2 mmole of I and 0.9 mmole of II by the action of mesitylenesulfonyl chloride (10.8 mmole) in 3.65 ml of absolute pyridine was carried out as described in [1] at room temperature for 20 h. The reaction mixture was cooled to −10°, and 8 ml of water, 14 ml of 50% triethylamine in pyridine, and 24 ml of 2 M ammonium hydroxide were added successively. The solution was neutralized to pH 7 by the addition of Dowex-50 (Py^+) resin, the mixture was filtered, the resin was washed with 20% aqueous pyridine, and the washings were combined; the filtrate was evaporated to 40 ml, and the residue was extracted four times with ether (80-ml portions). The aqueous solution was evaporated, diluted with water to 100 ml, and dialyzed in cellophane bags against 1.5 liter of distilled water (the water was changed three times every 24 h). Subsequent fractionation was accomplished with a column filled with Sephadex G-75 and 1 N ammonium carbonate buffer at a rate of 58 ml/h. Fractions were selected every 15 min. The eluted substances (12%, 4.1% of the starting mixture of monomers) in fractions 16-40 were combined and evaporated to remove ammonium carbonate. The distribution coefficient of the mixture of oligomers isolated in this manner was 0.43, according to the results of rechromatography with a column filled with Sephadex G-75 (the volume of resin was 70 cm^3, the column height was 65 cm, and the buffer was 1 N ammonium carbonate solution). Subsequent fractionation with this same column was carried out in a similar manner. The isolated products were freed from salts with a column filled with Sephadex G-10 with elution with a 0.01 N ammonium carbonate buffer. The collected eluate was concentrated, and the carbonate residues were removed with a column filled with the acid form of Dowex resin.

The treatment of the PT with acetic anhydride and HCl did not differ from the treatment described in [1, 9].

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