The cyclization reaction was performed in methylene chloride (c 10^{-3} M) at room temperature for four days, using equivalent amounts of carbodiimide and N-hydroxysuccinimide. After the solvent had been driven off, the unchanged polar components were eliminated on a column of IRA-410 (OH^-) anion-exchange resin and Dowex 50 × 12 (H^+) cation exchange resin. On subsequent gel chromatography on a column containing Sephadex LH-20 (methanol) calibrated with respect to the initial tripeptide (VI), a peak was separated which corresponded to the desired monomer (VII). Yield 12%, mp 165-166°C. Product (I) obtained after the treatment of compound (VII) with HCl in acetic acid differed chromatographically and electrophoretically from the starting materials and had negaitve reactions for an aromatic amino group at a COOH group.

In the process of synthesis, the purity of all the substances obtained was checked by thin-layer chromatography on silica gel and by high-voltage electrophoresis on paper. The elementary analyses of the substances corresponded to the calculated figures.

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SYNTHESIS OF NUCLEOTIDYL- AND OLIGONUCLEOTIDYL-(P → N)-LYSINES AND THEIR IMMOBILIZATION ON SEPHAROSE

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UDC 576.1

Immobilized nucleotides, oligonucleotides, and nucleic acids are widely used for the isolation of proteins [1] and nucleic acids [2]. Various methods have been proposed for the covalent fixing of nucleotides to a polymeric matrix: through a heterocyclic base [3] or a sugar [3, 4], and more rarely through a phosphoric acid residue [4–6]. The immobilization of oligonucleotides through the internucleotide phosphorus has not been described. Immobilized oligonucleotides in which the terminal groups and bases are accessible may have a high affinity for enzymes of the nucleic acid metabolism.

In this communication we consider the synthesis of α- and ε-lysine phosphoramides of nucleotides and dinucleoside phosphates, and also the immobilization of the latter compounds on Sepharose through the lysine residue.

The ethyl esters of thymidylyl-(5' → N^{α})-DL-lysine (I), thymidylyl-(5' → N^{ε})-DL-lysine (II), adenylyl-(5' → N^{α})-DL-lysine (III), and adenylyl-(5' → N^{ε})-DL-lysine (IV) were synthesized by the pyrophosphate (PP) [7] and the dicyclohexylcarbodiimide (DCC) [8] methods and through the mixed anhydrides with mesitylenecarboxylic acid [9]. In all cases, the α- and ε-amino groups of the lysine took part in the reaction with the nucleotide. On using the PP and DCC methods, the predominant product was the derivative at the α-amino group of the lysine. The yields of the ethyl esters of the nucleotidyl-(5' → N^{α})-DL-lysines were 70–80%, and of their ε-analogs 5–10%. By the mixed anhydride method, varying the pH of the medium, it was possible to obtain the ε-lysine derivatives as the main products. By this method, using a buffer with pH 9.5, we synthesized adenylyl-(5' → N^{α})-DL-lysine (V) and adenylyl-(5' → N^{ε})-DL-lysine (VI) with yields of 25 and 60%, respectively. The reaction mixtures were separated successively by chromatography and electrophoresis on paper.

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In the ethanol–0.5 M ammonium acetate (5:2) system, the R_f values of compounds (I-VI) were 0.75, 0.81, 0.65, 0.70, 0.15, and 0.18, respectively. The structures of the compounds obtained were confirmed by their acid (6 N HCl, 105°C, 24 h) hydrolysis followed by a determination of the base: phosphorus: lysine ratio. In all cases, it was close to 1:1:1.

The ethyl esters of thymidylyl-(3' → 5')-thymidine-($P^{i*} \rightarrow N^{\alpha}$)-DL-lysine (VII) and of thymidylyl-(3' → 5')-thymidine-($P^{i} \rightarrow N^{\varepsilon}$)-DL-lysine (VIII) were synthesized by the PP method which we have described previously [7]. The mixture of compounds (VII) and (VIII) obtained was isolated by chromatographing the reaction mixture on silica gel plates in the chloroform–methanol (9:1) system. The band with R_f 0.53 was eluted with chloroform–ethanol (1:1). The eluate was concentrated and the ethyl esters of the oligonucleotidyl-($P^{i} \rightarrow N$)-lysines were separated by preparative paper electrophoresis in a triethylammonium bicarbonate buffer with pH 7.5. The yields of compounds (VII) ($U_{rel.pT} = -0.1$) and (VIII) ($U_{rel.pT} = 0$) were 25 and 17%, respectively. Compounds (I-VIII) were immobilized on cyanogen-bromide-activated Sepharose 4B. Compounds (I-VIII) (4-6 μmole) were each dissolved in 0.5 ml of 0.1 M bicarbonate buffer, pH 9.5, and then 1 ml of activated Sepharose was added and the mixture was stirred at 4°C for 20 h. The Sepharose was filtered off and was washed with 15 ml of 0.1 M bicarbonate buffer, 25 ml of 0.1 M KCl, and 25 ml of water. The amount of covalently fixed nucleotides and oligonucleotides was determined by acid hydrolysis of 0.1 ml of the modified Sepharose (0.5 N HCl, 15 min, 90°C) followed by UV measurements in the filtrates. In this way it was found that 21, 17, 12, 6, 8, 12, and 11 μmoles of compounds (I, II, and IV-VIII), respectively, had been immobilized on 1 g of Sepharose. It can be seen from the experimental results that the esters of the nucleotidyl- and oligonucleotidyl-(P → N)-lysines are immobilized practically equally well through the α- and through the ε-amino group of lysine. Compounds (V) and (VI) having free carboxy groups, are immobilized less effectively.

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