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HYDROPHILIC POLYMERS FOR THE REVERSIBLE IMMOBILIZATION OF OLIGONUCLEOTIDES

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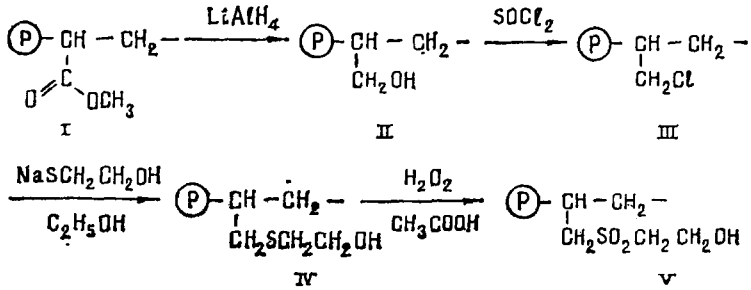
The aim of the present work was to create a hydrophilic polymer suitable for the reversible immobilization of oligonucleotides and for the development of methods for attaching oligonucleotides to a solid phase.

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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The immobilization of the oligonucleotides was best carried out on the polymer previously oxidized to form β-hydroxyethyl sulfone groups, since in this case the oligonucleotide chains can readily be removed by a β-elimination mechanism without subjecting the oligonucleotides to the action of an oxidant.

The polymers obtained had a capacity with respect to the anchoring groups of 0.2-0.4 mmole/g. Nonspecific sorption amounted to 0.3 OU/g of polymer for a mononucleotide and to 2 OU/g for poly(A). For celluloses of various types, the nonspecific sorption of poly(A) is usually between 5 and 20 OU/g [4].

Immobilization was investigated for the cases of dpT and dpG in 0.5 M MES buffer (pH 6.0) under the action of a water-soluble carbodiimide. The concentration of mononucleotide and carbodiimide in the experiments were 1-2 and 240 μmole/ml, respectively. The ratio of the amounts of support being studied to the buffer solution wetting it (0.8-1.3 g/ml) was considerably lower than when cellulose was used as the support, for which this ratio is 2 g/ml [5]. The immobilization was performed at room temperature for two days, and then, after the addition of a new portion of carbodiimide, for another two days. The loading of the monomer per 1 g of sorbent amounted to 30-120 OU of mononucleotide or 3-12 μmole/g.

The immobilization of oligonucleotides was carried out under the same conditions, and the extent of addition fell by approximtely an order of magnitude, amounting to 0.25 μmole/g for an eight-membered oligodeoxyribonucleotide 3'-phosphate and 0.60 μmole/g of polymer for a 15-membered oligonucleotide 5'-phosphate.

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