current of 7.5 W (initial conditions – 500 V, 14 mA; final conditions – 1100 V, 6.5 mA). The paper replica method [8] was used to locate the proteins. After the completion of the process the gel was divided into 38 sections and the proteins were eluted with 8 M urea solution. A component with pI 6.1 was readily detected visually, because of its high protein content, in the form of a semitransparent zone. Gradient SDS electrophoresis in 12.5-17.5% polyacrylamide gel using Laemmli's system [9] in the presence of 2-mercaptoethanol showed that the polypeptide that had been isolated was a monomer with a molecular weight of 21,600. Threonine was found as the NH_2-terminal amino acid. The UV spectrum of the polypeptide in 0.5% sodium dodecyl sulfate solution was characterized by an adsorption maximum at 276 nm. The amino acid composition of the protein was typical for zein polypeptides.

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REVERSIBLE IMMOBILIZATION OF OLIGONUCLEOTIDES ON NITROBENZYL-CONTAINING SUPPORTS

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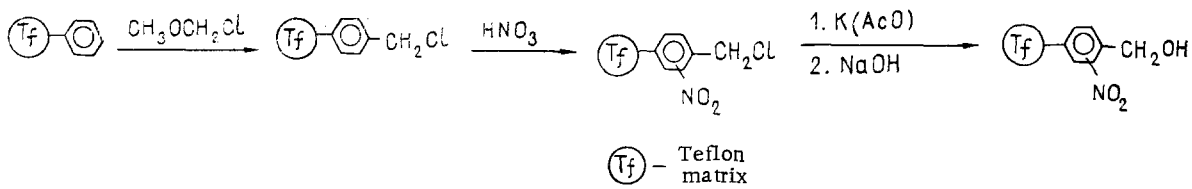
Oligonucleotides immobilized on insoluble supports are being used successfully for the isolation of individual nucleic acids [1], for the study of the interaction between nucleic acids [2], and for the genetic analysis of specific DNA sequences [3].

For the reversible immobilization of oligonucleotides we have used a polymeric support containing a O-nitrobenzyl anchoring group. A unique property of the O-nitrobenzyl group is the possibility of its elimination in an aqueous or organic medium on mild UV irradiation [4, 5]. As the polymeric matrix we used the modified polystyrene grafted onto the surface of inert polytetrafluoroethylene (Teflon) developed previously, which has well recommended itself. Below we give the scheme of obtaining a Teflon polymer of the grafted type containing O-nitrobenzyl groups. Each stage of the reaction was monitored by IR spectrophotometry. The use of the O-nitrobenzyl group for anchoring enables immobilization to be effected through the terminal phosphate groups of oligonucleotides while the functional groups of the heterocycles remain free.



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As an example of the covalent attachment and the elimination under the action of UV irradiation of di- and trideoxyribonucleotides, we investigated the possibility of using modified polystyrene with O-nitrobenzyl anchoring groups for the reversible immobilization of mono- and oligonucleotides.

Immobilization was performed similarly to [2] in 0.5 M MES buffer (pH 6.0), under the action of the water-soluble N-cyclohexyl-N'-[β-(4-methylmorpholinio)ethyl]carbodiimide toluenesulfonate, on a polymeric support, previously washed with ethanol, at 4°C for 3 days. The concentrations of oligonucleotides and the carbodiimide were 1-2 and 240 μmole/ml, respectively. Details of the charging of the polymer with the oligonucleotides and of the yields of products split out under the action of UV irradiation are given below:

Oligonucleotide	Charge, μmole/g	Split out, %
d(pCCA)	2.0	75
d(pACG)	2.68	62
d(pCC)	0.8	89

The photochemical removal of the nucleotide material from the polymer was performed with the aid of a DRSh 125 lamp at a distance of 5 cm from the sample, the time of irradiation being 40 min. A filter transmitting UV light with a wavelength greater than 310 nm was placed between the lamp and the sample in order to prevent UV cross-linkages at the heterocyclic bases. The amount of oligodeoxyribonucleotide split out was determined spectrophotometrically.

The homogeneity of the oligonucleotides after they had been split out under the action of UV irradiation was confirmed by high-performance ion-exchange chromatography.

Thus, the basic possibility of using a modified polystyrene polymer grafted onto Teflon and containing O-nitrobenzyl anchoring groups for the reversible immobilization of oligodeoxyribonucleotides has been demonstrated.

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