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Note

Reversed-phase high-performance liquid chromatography of very long oligodeoxyribonucleotides

MICHAEL HUMMEL, HERMANN HERBST* and HARALD STEIN
Institute of Pathology, Klinikum Steglitz, Free University Berlin, Hindenburgdamm 30, 1000 Berlin 45 (F.R.G.)
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The rapid development of automated DNA synthesis has facilitated the production of oligodeoxyribonucleotides (oligonucleotides) of up to 180 bases in length, that are predominantly used for gene synthesis[1,2]. While it is possible for some applications to use the crude product of synthesis directly, *e.g.*, as hybridization probes or sequencing primers, gene synthesis requires oligonucleotides conforming precisely to the desired sequence, since otherwise expensive and time-consuming site-specific mutagenesis procedures become necessary. During synthesis the individual coupling and deprotection steps are not completely quantitative, the crude product of synthesis containing, among other by-products, varying amounts of shorter oligonucleotides. Similarly, depurination under acidic conditions and strand scission in ammonia also lead to smaller sequences which need to be separated from the desired sequence by means of an high resolution technique.

Various high-performance liquid chromatography (HPLC) procedures have been reported for the purification and separation of oligonucleotides. However, most of these techniques are restricted to oligonucleotides of 45–60 bases in length[3–8]. Paired-ion-exchange chromatography is capable of separating very long detritylated oligonucleotides, but requires corrosion-resistant equipment and subsequent desalting[9]. The alternative, and previously the only technique applicable to longer oligonucleotides, is electrophoretic separation of the crude mixture by polyacrylamide gel electrophoresis (PAGE) followed by electroelution[10,11]. The latter procedures have some drawbacks, as they are labour intensive and time consuming, show a low yield due to incomplete recovery from the gel and may require radioactive labelling. In addition, PAGE does not lend itself to automation and large scale preparations. Here we report a reversed-phase (RP) HPLC procedure for the purification and separation of very long 5′-O-dimethoxytrityl(DMT)-derivatized oligonucleotides, and demonstrate its resolving power for several oligonucleotides of 88–143 nucleotides in length.

EXPERIMENTAL

Equipment

Five oligodeoxyribonucleotides (Table I) were generated by solid phase phosphoramidite chemistry on an Applied Biosystems (Weiterstadt, F.R.G.) Model 381A

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