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Note

Fractionation of synthetic deoxyribopolynucleotides on silica gel thin-layer plates*

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In the field of deoxyribopolynucleotide synthesis and sequencing, one of the major problems is the requirement of a simple and versatile method for the fractionation and characterization of these compounds. The recent successful application of two-dimensional thin-layer chromatography (TLC) on Avicel-cellulose plates[1,2] for the fractionation of complex reaction mixtures of synthetic oligonucleotides led us to attempt the fractionation of the higher oligonucleotides on silica gel plates. Previous work by others[3–5] has indicated the potential usefulness of these plates. In the present studies, we have now exploited the silica gel TLC using commonly available aqueous solvent systems for the rapid resolution and identification of synthetic oligonucleotides up to decanucleotides. In order to demonstrate the usefulness of silica gel plates, we have particularly selected one of the most complex reaction mixtures, *i.e.* chemically polymerized thymidine 5'-phosphate as a model system.

EXPERIMENTAL

Analytical precoated plates of silica gel 60 F_{254} (E. Merck, Darmstadt, G.F.R.) (0.25 mm) on glass (20 × 20 cm) were supplied by Brinkmann, Westbury, N.Y., U.S.A.

The chemical polymerization of thymidine 5'-phosphate was carried out with mesitylenesulfonyl chloride (1.5 mequiv.) in anhydrous pyridine solution[6].

The following solvent systems were used: Solvent I, *n*-propanol–concentrated ammonium hydroxide–water (60:20:20); Solvent II, isobutyric acid–1 *M* ammonium hydroxide–0.1 *M* EDTA (100:60:1.6).

RESULTS AND DISCUSSION

The two-dimentional TLC pattern of polymerized thymidine 5'-phosphate (5–10 A_{260} units) on silica gel in Solvents I and II is shown in Fig. 1. The identification of each spot was carried out by: (i) Rechromatography on TLC plates after alkaline bacterial phosphatase treatment; (ii) degradation of the dephosphorylated compounds with snake venom or spleen phosphodiesterase enzymes; (iii) comparison of mobilities with known samples on TLC under different solvent systems; and (iv)

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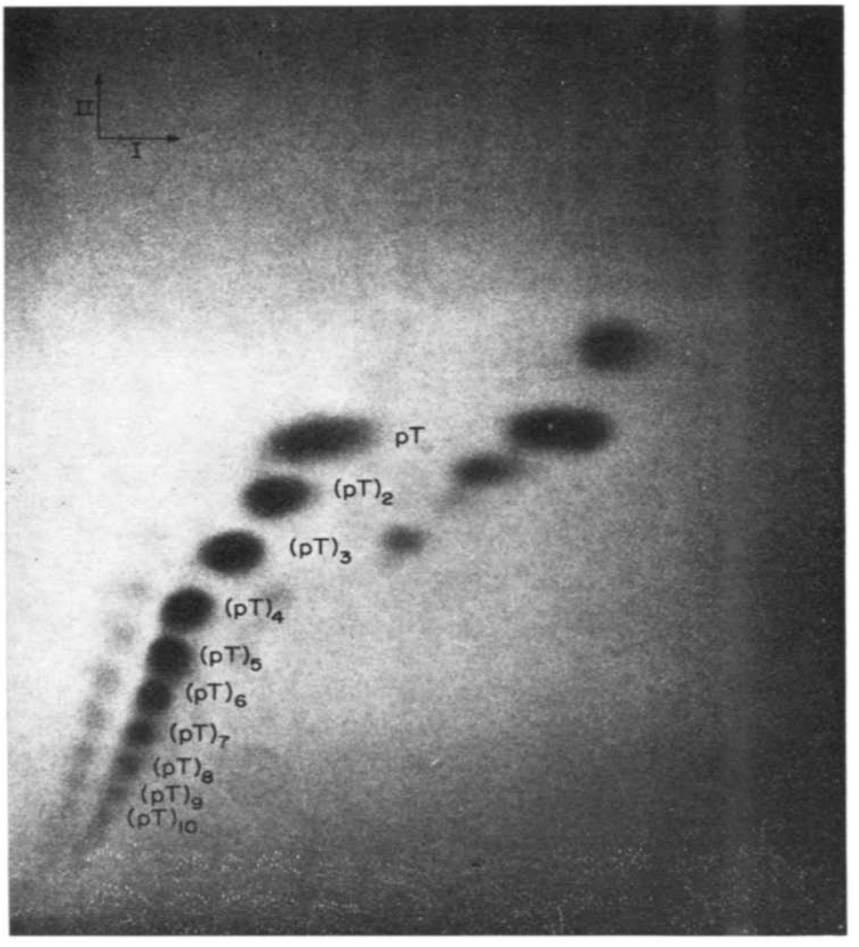

Fig. 1. Two-dimensional TLC fractionation of chemically polymerized thymidine 5'-phosphate. Solvent I was used in the first dimension and Solvent II in the second dimension.

examination of their UV spectra. A very important and unique pattern of the position of various spots on the chromatographic plate has been observed. For example, all the linear homologues (pT) to $(pT)_{10}$ occupy a middle row in the chromatogram. The cyclic homologues fall on the right-hand row. Finally the third homologous series containing C-pyridinium thymidine and a terminal phosphate group were detected in the left-hand row. Thus the separation of this reaction mixture by two-dimensional TLC on silica gel plates, using commonly available solvent systems, into three different rows indicates its great potential.

In conclusion the silica gel plate for TLC using the aqueous solvent systems offers the following advantages: (i) It can be used as a very quick analytical tool to check the purity of the oligonucleotide up to decanucleotide by using as low as 0.1 A_{260} unit; (ii) the resolution of the individual components is generally more rigorous into well defined spots; (iii) it is less time-consuming as compared to paper and column

chromatography; (iv) thus recovery of the compounds is much better than by column chromatography.

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