The Behaviour of Oligodeoxynucleotides on Thin-Layer Chromatography on Polyethyleneimine-Cellulose and Ion-Exchange Paper Electrophoresis

APPLICATIONS IN FRACTIONATING AND SEQUENCING TERMINALLY LABELLED OLIGODEOXYNUCLEOTIDES

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(Received 17 October 1973)

The electrophoretic behaviour of oligodeoxynucleotides on ion-exchange papers was studied systematically with particular attention to applications in sequence analysis. Complex mixtures of larger oligonucleotides were fractionated by electrophoresis on cellulose acetate followed by t.l.c. on polyethyleneimine-cellulose. The behaviour of the oligonucleotides on polyethyleneimine-cellulose and on the two-dimensional system can provide a useful guide to their sequence. Detailed results from some of these experiments have been deposited as Supplementary Publication SUP 50031 (13 pages) at the British Library (Lending Division) (formerly the National Lending Library for Science and Technology), Boston Spa, Yorks. LS23 7BQ, U.K., from whom copies can be obtained on the terms indicated in Biochem. J. (1973), 131, 5.

Terminal-labelling methods have been used to introduce radioactive atoms of very high specific radioactivity into precise regions of DNA to facilitate their isolation and sequence determination. Examples include sequences at the termini of bacteriophage DNA (Murray & Murray, 1973; Weigel et al., 1973) and sites of cleavage of DNA by restriction enzymes (Kelly & Smith, 1970; Hedgpeth et al., 1972; Bigger et al., 1973). Progress in this area depended on the methods available for fractionating and sequencing oligodeoxynucleotides.

Electrophoresis on DEAE-cellulose paper was used in separation and sequencing of micro-quantities of uniformly $^{32}$P-labelled oligoribonucleotides (Sanger et al., 1965). Small oligoribonucleotides were partially digested with exonuclease and the resulting series of products was fractionated by DEAE-cellulose paper electrophoresis. The composition of these partial digestion products could be found by digesting them completely with exonuclease or alkali, and hence the sequence of the parent oligonucleotide could be deduced. But it was also found that the sequence of an oligonucleotide could be deduced from the relative positions of its partial digestion products on DEAE-cellulose paper, since the relative change in mobility of an oligonucleotide on addition (or subtraction) of a mononucleotide (i.e. $M$ value) was characteristic of that mononucleotide.

For a terminally labelled oligonucleotide, deduction of its sequence from $M$ values is the only method possible, because the composition of its partial digestion products cannot be ascertained by complete digestion with exonuclease as with a uniformly labelled oligonucleotide.

The behaviour of oligodeoxynucleotides on DEAE-cellulose paper is similar to that of equivalent oligoribonucleotides (Murray & Offord, 1966; Murray, 1970), but the unambiguous deduction of the sequence of a terminally labelled oligonucleotide from $M$ values is not possible, because G and T residues cannot be distinguished in this system. However, electrophoresis on AE (aminoethyl)-cellulose paper at pH 3.5 (Murray, 1973) distinguishes the $M$-value of G from that of T, and the two systems together provide the information to deduce the sequence of a terminally labelled oligonucleotide without ambiguity.

Electrophoresis of partial digests on DEAE-cellulose paper alone is sufficient to determine the sequence of oligoribonucleotides derived from digestion of RNA with RNAase (ribonuclease) $T_1$ or pancreatic RNAase, because the base specificities of these endonucleases ensure that the oligonucleotides contain only one G residue or one pyrimidine respectively, which is 3'-terminal in each case.

These methods, however, are limited to relatively small oligonucleotides (five to ten residues long, depending on composition). Larger oligoribonucleotides have been fractionated by t.l.c. on polyethyleneimine-cellulose (Southern & Mitchell, 1971; Griffin, 1971) and by ‘homochromatography’ on thin layers of DEAE-cellulose (Brownlee & Sanger, 1969; Ling, 1972). We have explored other fractionation conditions with ion-exchange papers by varying the pH
and molarity of the electrophoresis buffers, to see whether the size limits of nucleotide which these systems can fractionate could be improved, or if one single system could replace the two systems, while still retaining unambiguous $M$ values for each mononucleotide. Also, following the work of Southern & Mitchell (1971), we have examined the use of t.l.c. on polyethyleneimine-cellulose for fractionating oligodeoxyribonucleotides and deducing their sequences from $M$ values.

In evaluating fractionation systems, it is necessary to have nucleotides of suitable size and known sequence. Nuclease digests of DNA have usually been used previously, but their complexity causes difficulties. We have used the homologous oligodeoxyribonucleotide series oligo(dT), oligo(dA), oligo(dC), and oligo(dG) as model nucleotides.

Abbreviations

Since only deoxynucleotides are discussed in this paper, A is used for deoxyadenyllic acid, G for deoxyguanylic acid etc. All the nucleotides referred to in the text, Plates, Figures and Tables have a 5'-phosphate, unless stated otherwise, which has been omitted throughout.

Materials and Methods

Materials

Cellulose acetate strips were from Oxo Ltd., London E.C.4, U.K.; ion-exchange papers (AE-81 and DE-81) were Whatman Chromedia, Whatman Biochemicals, Maidstone, Kent, U.K.; polyethyleneimine-cellulose layers (Polygram CEL 300 PEI), manufactured by Macherey Nagel and Co., Düren, Germany, were obtained through Camlab (Cambridge) Ltd., Cambridge, U.K., and were washed by ascending chromatography in the following series of solvents: 1.5M-formic acid adjusted to pH3.7 with pyridine (two washes if necessary), methanol (to improve flow rates) and water. The dye markers Xylene Cyanol FF (blue), Acid Fuchsin (red), Orange G (yellow) were from G. R. Gurr Ltd., London S.W.6, U.K.

Pancreatic deoxyribonuclease I, snake venom phosphodiesterase and bacterial alkaline phosphatase were from Worthington Biochemical Corp., Freehold, N.J., U.S.A.; polynucleotide kinase was prepared from Escherichia coli infected with bacteriophage T4 by the procedure of Richardson (1965), with the modification of Wu & Kaiser (1968), as described by Murray (1973).

$[\gamma^{32}P]_{\text{ATP}}$ was from The Radiochemical Centre, Amersham, Bucks., U.K. The copolymers poly(dA)-poly(dT) and poly(dG)-poly(dC) were from Miles Seravac (Pty) Ltd., Maidenhead, Berks., U.K.

Dinucleoside monophosphates were from Sigma Ltd., Norbiton Station Yard, Kingston-upon-Thames, Surrey, U.K.

Methods

Details of electrophoresis, radioautography and micro techniques for nucleotide analysis are as described by Sanger et al. (1965) and Murray (1970). Mobilities of compounds are given with respect to the blue dye (xylene cyanol FF) unless otherwise stated. Polyethyleneimine-cellulose chromatography was largely as described by Randerath & Randerath (1967) and Southern & Mitchell (1971).

Preparation of terminally labelled homo-oligodeoxyribonucleotides. Poly(dA)-poly(dT) and poly-(dG)-poly(dC) were dissolved in 20mM-Tris·HCl (pH7.5)-1mM-MgCl$_2$, and dialysed against the same buffer.

The copolymers were partially digested with deoxyribonuclease I (Bollum, 1965). Reaction mixtures containing 0.1-0.4mg of copolymer/ml, 0.1–10μg of deoxyribonuclease I/ml, 5mM-MgCl$_2$ and 20mM-Tris·HCl, pH7.5, in a total volume of 20μl were incubated for 10–30min at 37°C in a capillary tube. The terminal 5'-phosphates of the digestion products were removed with phosphatase: 5μl of a solution containing 50mM-EDTA and 0.2mg of bacterial alkaline phosphatase/ml, was added and the mixture incubated for 1h at 37°C. The phosphatase was removed by absorption on to phosphocellulose paper (Szekely & Sanger, 1969). The phosphocellulose paper was pre-washed in dilute NH$_3$ solution and rinsed in water. The copolymer digests were applied to phosphocellulose paper pennants, which were allowed to dry and eluted with water into a capillary tube. The eluates were evaporated to dryness on polythene in a vacuum desiccator.

Polynucleotide kinase was used to transfer $[^{32}P]_{\text{-}}$phosphate groups to the 5'-termini of the oligonucleotides (Richardson, 1965). The dephosphorylated oligonucleotides were dissolved in 20μl of a solution containing 0.1m-Tris·HCl, pH7.5, 10mM-MgCl$_2$, 20μM-mercaptoethanol, 0.5 unit of polynucleotide kinase (Richardson, 1965) and 0.1m-[γ-32P]ATP (specific radioactivity approx. 14Ci/mmol) and incubated for 30min at 37°C.

The labelled copolymer digests, prepared as described above, were used in most experiments. Electrophoresis on cellulose acetate (pH3.5, 7m-urea) in the first dimension, followed by paper electrophoresis or t.l.c. on polyethyleneimine-cellulose, separated the homo-oligodeoxyribonucleotide series derived from either copolymer.

Sometimes it was necessary to purify the homooligomers from undigested polynucleotides and low-molecular-weight contaminants such as ATP and P$_i$. The digests were first desalted by applying them to
DEAE-cellulose paper pennants [which had been washed previously in 30% (w/v) triethylamine carbonate] washing with water, and eluting with 30% triethylamine carbonate. The triethylamine carbonate was removed by evaporation in a vacuum desiccator, and the nucleotides were applied in a volume of about 3 μl, as a 0.5 cm streak, to cellulose-acetate strips and electrophoresed (pH3.5, 7M-urea; 100 V/cm, 10 min). The nucleotides were transferred to polyethyleneimine-cellulose layers which were developed in pyridine-formate buffers, pH3.7, the thin layer being transferred during chromatography to solvents of progressively increasing concentrations of formic acid as follows: for poly(dG)-poly(dC) digests, 0.75, 1.5, 2.5 and 3.0M; for poly(dA)-poly(dT) digests, 1.2, 1.5, 2.5, and 3.0M. Each homo-oligodeoxyribonucleotide series was eluted.

Preparation of di-deoxyribonucleoside monophosphates. A solution (10 μl) containing 0.2 μg of uniformly \( ^{32}P \)-labelled bacteriophage lambda DNA (7.5 × 10^5 c.p.m.), 8 mM MnCl₂ and 1 mg of deoxyribonuclease I/ml was incubated at 37°C for 1 h and fractionated on cellulose acetate (pH3.5) and then polyethyleneimine-cellulose (in pyridine-formate, 1.5M, pH3.7). The main products of this digestion were 5'-phospho-dinucleotides. The spots were located by radioautography, eluted, electrophoresed in some cases on AE-cellulose paper to separate sequence isomers, and treated with phosphatase to remove the terminal phosphates (10 μl of solution containing 0.1 mg of bacterial alkaline phosphatase/ml and 0.1M-Tris-HCl, pH7.5 was incubated at 37°C for 1 h). Their identities were deduced from (i) the positions of the spots on the cellulose acetate/polyethyleneimine-cellulose 'map', (ii) the mobilities of some of the parent dinucleotides on AE-cellulose paper (pH3.5), and (iii) digestion with venom exonuclease and electrophoresis of the products on AE-cellulose paper, pH3.5.

Elution from polyethyleneimine-cellulose layers. The following method (A. Mirzaberkov, unpublished work) was used. The spot, having been located by radioautography, was cut out and held, layer side down, to the pointed end of a 3MM paper pennant, by using flat-tipped forceps. The other end of the paper pennant was placed between two glass slides in an elution tray containing 30% triethylamine carbonate, so that liquid flowed along it and through the polyethyleneimine-cellulose spot. The forceps were then removed and a glass capillary was used to collect the liquid from the tip of the paper pennant.

Results and Discussion

Use of homo-oligodeoxyribonucleotides in sequencing investigations

Nuclease digests of RNA and DNA, and Burton digests (Burton & Peterson, 1960) of DNA (pyrimidine tracts) have been used previously to investigate systems for fractionating oligonucleotides (Sanger et al., 1965; Murray, 1970; Southern & Mitchell, 1971). Endonuclease digests of DNA are unsuitable for evaluating methods of sequencing larger oligodeoxynucleotides, because they comprise very complex mixtures whose larger constituents (particularly sequence isomers) usually cannot be resolved. Burton digests can obviously only give information about pyrimidines.

![Fig. 1. Electrophoresis of homo-oligodeoxyribonucleotides on AE-cellulose paper at pH3.5 (a) and DEAE-cellulose paper at pH2.0 (b)](image)

Electrophoresis buffers were: 10% (v/v) acetic acid-1% (v/v) pyridine (pH3.5) for AE-cellulose paper, and 8.75% (v/v) acetic acid-2.5% (v/v) formic acid (pH2.0) for DEAE-cellulose paper. The fastest moving spot is in each case the mononucleotide. b, blue marker dye.
Copolymer digests give relatively long homo-oligodeoxynucleotides whose sequences are, of course, known. These can be used to distinguish effects due to composition from effects due to size. However, their behaviour when fractionated on a system might not vary to the same extent as that found with oligodeoxynucleotides of random sequence.

The behaviour of homo-oligomers was examined in established systems used in sequencing, i.e. electrophoresis on AE-cellulose paper and DEAE-cellulose paper (Fig. 1). An oligonucleotide can be sequenced by fractionating its partial nuclease digest with these two systems (Murray, 1973), because \( M \) values [the ratio of the distance between two oligonucleotides that differ by the addition (or loss) of one mononucleotide, to the distance from the origin of the larger oligonucleotide], are specific for the mononucleotide by which the two oligonucleotides differ.

\( M \) values for most of the homo-oligomers (Table 1) lie within the limits found for values from partial digests of oligodeoxynucleotides from a digest of DNA (Table 2). Table 1 also reveals that \( M \) values usually increase as nucleotides get longer and shows the necessity of using the two systems together to confirm the sequence of a nucleotide.

Fig. 1 shows the limitations in sizes of nucleotides that can be fractionated on these paper electrophoresis systems. G residues are particularly retarding and \((dG)_n\) is the largest member of the oligo(dG) series which can be conveniently fractionated on AE-cellulose paper.

The mobilities of dinucleoside monophosphates on the two paper electrophoresis systems are given in Table 3.

![Table 1. \( M \) values for homo-oligodeoxynucleotides on AE-cellulose and DEAE-cellulose papers](image)

<table>
<thead>
<tr>
<th>Homo-oligonucleotides</th>
<th>AE-cellulose ( (\text{pH}3.5) )</th>
<th>DEAE-cellulose ( (\text{pH}2.0) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A_2-A_3 )</td>
<td>1.13</td>
<td>0.28</td>
</tr>
<tr>
<td>( A_3-A_4 )</td>
<td>1.68</td>
<td>0.47</td>
</tr>
<tr>
<td>( A_4-A_5 )</td>
<td>1.57</td>
<td>0.54</td>
</tr>
<tr>
<td>( A_5-A_6 )</td>
<td>1.90</td>
<td>0.60</td>
</tr>
<tr>
<td>( A_6-A_7 )</td>
<td>0.80</td>
<td>0.80</td>
</tr>
<tr>
<td>( G_2-G_3 )</td>
<td>2.74</td>
<td>1.93</td>
</tr>
<tr>
<td>( G_3-G_4 )</td>
<td>3.04</td>
<td>1.81</td>
</tr>
<tr>
<td>( G_4-G_5 )</td>
<td>2.20</td>
<td>2.20</td>
</tr>
<tr>
<td>( C_2-C_3 )</td>
<td>0.40</td>
<td>0.03</td>
</tr>
<tr>
<td>( C_3-C_4 )</td>
<td>0.54</td>
<td>0.03</td>
</tr>
<tr>
<td>( C_4-C_5 )</td>
<td>0.92</td>
<td>0.04</td>
</tr>
<tr>
<td>( C_5-C_6 )</td>
<td>1.30</td>
<td></td>
</tr>
<tr>
<td>( T_2-T_3 )</td>
<td>1.17</td>
<td>2.26</td>
</tr>
<tr>
<td>( T_3-T_4 )</td>
<td>1.47</td>
<td>2.38</td>
</tr>
<tr>
<td>( T_4-T_5 )</td>
<td>1.71</td>
<td>2.30</td>
</tr>
<tr>
<td>( T_5-T_6 )</td>
<td>1.50</td>
<td></td>
</tr>
</tbody>
</table>

![Table 2. \( M \) values calculated from partial nuclease digests of nucleotides from DNA digests (Murray, 1970, 1973)](image)

<table>
<thead>
<tr>
<th>Mononucleotide added</th>
<th>AE-cellulose ( (\text{pH}3.5) )</th>
<th>DEAE-cellulose ( (\text{pH}2.0) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A )</td>
<td>0.9-1.5</td>
<td>0.4-1.0</td>
</tr>
<tr>
<td>( G )</td>
<td>2.0-3.0</td>
<td>1.4-3.0</td>
</tr>
<tr>
<td>( C )</td>
<td>0.5-0.7</td>
<td>0-0.3</td>
</tr>
<tr>
<td>( T )</td>
<td>1.0-1.5</td>
<td>1.4-3.0</td>
</tr>
</tbody>
</table>

![Table 3. Mobilities of dinucleoside monophosphates on DEAE-cellulose paper \( (\text{pH}2.0) \) and AE-cellulose paper \( (\text{pH}3.5) \)](image)

Mobilities are expressed with respect to blue dye (Xylene Cyanol FF). Electrophoresis buffers were as described in the legend to Fig. 1.

<table>
<thead>
<tr>
<th>Di-ribonucleoside monophosphate</th>
<th>Mobility on DEAE-paper</th>
<th>Mobility on AE-paper</th>
<th>Di-deoxynucleoside monophosphate</th>
<th>Mobility on AE-paper</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{ApA} )</td>
<td>0.93</td>
<td>0.76</td>
<td>( \text{ApA} )</td>
<td>0.84</td>
</tr>
<tr>
<td>( \text{ApG} )</td>
<td>1.66</td>
<td>0.77</td>
<td>( \text{ApG} )</td>
<td>0.90</td>
</tr>
<tr>
<td>( \text{ApC} )</td>
<td>0.79</td>
<td>0.65</td>
<td>( \text{ApT} )</td>
<td>1.12</td>
</tr>
<tr>
<td>( \text{ApU} )</td>
<td>2.35</td>
<td>1.04</td>
<td>( \text{GpA} )</td>
<td>0.96</td>
</tr>
<tr>
<td>( \text{GpA} )</td>
<td>—</td>
<td>0.85</td>
<td>( \text{GpG} )</td>
<td>0.85</td>
</tr>
<tr>
<td>( \text{GpC} )</td>
<td>1.75</td>
<td>0.93</td>
<td>( \text{GpT} )</td>
<td>1.12</td>
</tr>
<tr>
<td>( \text{GpU} )</td>
<td>2.00</td>
<td>0.99</td>
<td>( \text{CpA} )</td>
<td>0.96</td>
</tr>
<tr>
<td>( \text{CpA} )</td>
<td>0.79</td>
<td>0.63</td>
<td>( \text{CpG} )</td>
<td>0.93</td>
</tr>
<tr>
<td>( \text{CpG} )</td>
<td>1.67</td>
<td>0.88</td>
<td>( \text{CpG} )</td>
<td>0.85</td>
</tr>
<tr>
<td>( \text{Cpc} )</td>
<td>0.81</td>
<td>0.89</td>
<td>( \text{CpT} )</td>
<td>1.18</td>
</tr>
<tr>
<td>( \text{CpU} )</td>
<td>2.46</td>
<td>1.15</td>
<td>( \text{TpA} )</td>
<td>1.19</td>
</tr>
<tr>
<td>( \text{UpA} )</td>
<td>2.40</td>
<td>1.12</td>
<td>( \text{TpG} )</td>
<td>1.14</td>
</tr>
<tr>
<td>( \text{UpG} )</td>
<td>2.05</td>
<td>0.99</td>
<td>( \text{TpT} )</td>
<td>1.41</td>
</tr>
<tr>
<td>( \text{UpC} )</td>
<td>2.46</td>
<td>1.16</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

1974
Homo-oligodeoxynucleotides were electrophoresed on AE-cellulose paper with buffers of varying molarities, but all adjusted to pH 3.5 with pyridine. The buffers contained: 10% acetic acid (0.09 M); 9.55% acetic acid, 0.9% formic acid (0.17 M); 9.1% acetic acid, 1.75% formic acid (0.25 M); and 8.75% acetic acid, 2.5% formic acid (0.32 M). The molarity refers to total anion concentration calculated by using the Henderson–Hasselbach equation. (a) shows M values for A (▲) and C (○); (b) shows M values for G (■) and T (○). Symbols joined by lines refer to M values for a given pair of homo-oligodeoxynucleotides. Lines of increasing M values refer to homo-oligodeoxynucleotides of progressively increasing size listed in Table 1.

Terminally labelled oligonucleotides were prepared by degrading DNA with deoxyribonuclease I, treating with phosphatase, labelling with polynucleotide kinase and [γ-32P]ATP, and fractionating the products by two-dimensional electrophoresis on DEAE-cellulose paper (Murray, 1973). The larger oligonucleotides were eluted, partially digested with venom exonuclease, and electrophoresed on AE-cellulose paper, with buffers containing 10% acetic acid, adjusted to various pH values with pyridine. (a) shows M values for A (▲) and C (○); (b) shows M values for T (○); 1 = TGC → TGCA; 2 = TG → TGA; 3 = GG → GGA; 4 = AG → AGA; 5 = TG → TGC; 6 = TGT → TGTT; 7 = TG → TGT; 8 = GC → GCT; 9 = TC → TCT; 10 = AG → AGT.
Effect of pH and concentration of buffer on electrophoretic behaviour of oligodeoxynucleotides

These were studied systematically to find conditions where the efficiency of these systems would be improved. However, it was found that, when mobilities of oligodeoxynucleotides are increased by raising the concentration of the electrophoresis buffers, the range of M values for the four mononucleotides is compressed, causing them to overlap each other (see Figs. 2, 3 and 4). Thus a significant increase in the mobilities of larger oligodeoxynucleotides in these systems can only be made at the expense of distinctive M values. Further, no conditions were found where a single fractionation would give reliably distinctive M values for all four mononucleotides. More detailed results of these experiments, including plots of mobilities and M values of oligonucleotides on AE81 paper as a function of pH and concentration of the electrophoresis buffer and of chain length of the oligonucleotide are deposited with the British Library (Lending Division) (formerly the National Lending Library for Science and Technology), Boston Spa, Yorkshire, LS23 7BQ, U.K.

Fractionation of deoxynucleotides on polyethyleneimine

Deoxyribonuclease I digests and Burton digests of DNA were fractionated by electrophoresis on cellulose acetate followed by chromatography on polyethyleneimine-cellulose layers (Southern & Mitchell, 1971).

Nucleotides fractionated in these experiments were eluted and analysed (Plate 1 and Fig. 2). Larger oligonucleotides were fractionated on polyethyleneimine-cellulose than by ion-exchange paper electrophoresis, and the system has the usual advantages of t.l.c.

The use of the two-dimensional fractionation methods in sequence analysis depends on the change in behaviour on addition of a mononucleotide to an oligonucleotide being characteristic and unique for each of the four mononucleotides. In the electrophoresis step, addition of a T or a G residue to an oligonucleotide is always accelerating at pH3.5, the effect of an A residue may be slightly accelerating or retarding, whereas the effect of a C residue is usually strongly retarding. The addition of C residues to some oligonucleotides already containing a high proportion of C residues may have an accelerating

Fig. 4. Effect of pH of the electrophoresis buffer (8.75% acetic acid-2.5% formic acid) on M values of oligodeoxynucleotides on DEAE-cellulose paper

Terminally labelled nucleotides were prepared as described in the legend to Fig. 3. The pH of the electrophoresis buffer was adjusted with pyridine. M values for G (•): 1 = (TG) → (TG)G; 2 = TAT → TATG. M values for T (○): 3 = TG → TGT; 4 = TA → TAT; 5 = GAC → GACT; 6 = GCC → GCCT. M values for A (▲): 7 = TGT → TGTGA. M Values for C (□): 9 is the mean of GC → GCC, TG → TGC, TGC → TGCC, and GA → GAC.

Fig. 5. Two-dimensional fractionation of pyrimidine tracts, labelled in their terminal 5'-phosphates

Calf thymus DNA was degraded with formic acid and diphenylamine (Burton & Peterson, 1960), treated with phosphatase, labelled by using polynucleotide kinase, and fractionated by electrophoresis on cellulose acetate, pH3.5, (7M-urea) and chromatography on polyethyleneimine-cellulose, with 1.5M-pyrimidine formate, pH3.7, as solvent.
EXPLANATION OF PLATE I
Two-dimensional fractionation of terminally labelled 5'-oligodeoxynucleotides by electrophoresis on cellulose acetate and chromatography on polyethyleneimine-cellulose layers

Labelled oligonucleotides were prepared by digesting DNA with deoxyribonuclease I, treating with phosphatase, and then labelling with polynucleotide kinase and [γ-32P]ATP. The digest was electrophoresed on cellulose acetate [in 5% (v/v) acetic acid–7M-urea, pH 3.5], at 100V/cm. The yellow dye was run about 40cm from the origin in this case to increase separation, and the nucleotides were transferred to two polyethyleneimine-cellulose layers. After developing with 1.5M-pyridine-formate, pH 3.7, the spots were located by radioautography, eluted, and their major components purified by electrophoresis on AE-cellulose paper (pH 3.5). Nucleotides were sequenced by electrophoresis of their partial venom digests on AE-cellulose paper, pH 3.5, and on DEAE-cellulose paper, pH 2.0. PEI-cellulose is polyethyleneimine-cellulose. The line drawing (ii) is a key to (i).

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The ratio of distance by which an oligonucleotide is retarded on polyethyleneimine-cellulose when another mononucleotide is added to it to the distance of the resulting oligonucleotide from the origin, i.e. M value, is plotted against the R_p of the resulting oligonucleotide. Measurements were taken from fractionations on polyethyleneimine-cellulose (with 1.5M-pyridine-formate, pH3.7) of homo-oligomers, pyrimidine tracts and nucleotides from the ends of bacteriophage DNA (Murray & Murray, 1973), which had been labelled in their 5'-phosphates by using polynucleotide kinase, and separated in the first dimension on cellulose acetate. ●, G; △, T; ■, A; ○, C.

Effect of varying the developing solvent

The effects of concentration and pH of the pyridine-formate solvent on the R_p values of homo-oligomers are shown in Figs. 7(a)-(d) and 8(a)-(c). Conditions may be varied to suit the mixture to be fractionated; for example, one can separate quite long nucleotides and resolve small ones on the same chromatogram by progressively increasing the concentration of the developing buffer.

Conclusion

Electrophoresis of partial exonuclease digests of terminally labelled oligodeoxynucleotides on AE-cellulose paper (pH3.5) and DEAE-cellulose paper

* M value is used here in chromatography in an analogous way to its use in electrophoresis.
(pH2.0) provides the most reliable method for sequencing smaller oligodeoxynucleotides. No equally good method applicable to larger terminally labelled oligodeoxynucleotides is yet available. The two-dimensional method using cellulose acetate and polyethyleneimine-cellulose, though not itself always dependable, has already proved valuable, and, in conjunction with other techniques, may provide enough information for the unequivocal determination of sequences.
Fig. 8. Effect of pH of 1.5M-pyridine-formate buffer on R_f values of homo-oligomers on polyethyleneimine-cellulose
(a) Oligo(dC) and oligo(dG); (b) oligo(dA); (c) oligo(dT).

We are grateful to Dr. R. W. Old and Dr. E. M. Southern for helpful discussions, and to the Science Research Council for financial support.

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