THE USE OF THIN ACRYLAMIDE GELS FOR DNA SEQUENCING

F. SANGER and A. R. COULSON
MRC Laboratory of Molecular Biology, Cambridge CB2 2QH, England

Received 13 January 1978

1. Introduction

The newer rapid methods for DNA sequencing [1–3] all use electrophoresis on acrylamide gels in denaturing conditions for the final sequence analysis, and the extent to which sequences can be read is often dependent on the degree of resolution that can be obtained with these gels. The gels are normally 1–2 mm thick and there is some spreading of the bands on the autoradiograph due to the fact that part of the radiation source is some distance from the film. One way of overcoming this is to use thinner gels and we have found that with gels of about 0.4 mm thickness sharper bands can be obtained, with a corresponding improvement in resolution. This simple modification in technique has a number of other advantages. Firstly, less material can be used so that one can economise both on the DNA used and the acrylamide. Secondly, it is possible to run the electrophoresis at higher voltages than is used with conventional gels. Using the plus and minus [1] and the terminator [3] methods, we have found serious variations in the distances between consecutive nucleotide bands in regions of dyad symmetry where base-paired loop structures can form, and this can lead to difficulties of interpretation. The effect can be overcome by running the gels at higher voltages so that the resultant higher temperatures in the gels cause complete denaturation of the loops. High temperatures may cause distortion of the bands and cracking of the glass plates, but these effects are less apparent with the thin gels than with the conventional ones.

Abbreviations: As this paper is concerned with DNA, the letters C, T, A and G are used for the deoxyribonucleotides. The prefix dd is used for the 2′,3′-dideoxy derivatives (e.g., ddATP, 2′,3′-dideoxyadenosine 5′-triphosphate) and the prefix Ara for the arabinose analogues

2. Materials and methods

The conditions for the terminator sequencing procedure were as described [3]. Spacers and well-forming templates for the thin acrylamide gels were made from 0.35 mm thick 'Plastikard' (Slater's (Plastikard) Ltd, Matlock Bath, Derbyshire) or, in earlier experiments, from two or three thicknesses of 0.2 mm polythene sheet. The electrophoresis was carried out in the apparatus [4] with minor modifications. An apparatus of this type is available from Raven Ltd (Haverhill, Suffolk).

The gels were formed between two 20 × 40 × 0.4 cm glass plates, one with a notch 16.5 × 2 cm cut out from the top to allow contact between the gel and the upper buffer compartment of the apparatus. The inside of this plate was siliconised with 'Repelcote' (Hopkin & Williams Ltd., Romford) to facilitate removal of the plate from the gel after the electrophoresis. The sides and bottom of the gel-former were sealed with waterproof electrical tape. It was found most convenient to pour the gel at an angle of about 45° from a pipette. After pouring, the well-former was inserted and the gel allowed to polymerize in a horizontal position. Slots were normally 0.75 cm deep. All solutions and buffer were as described [5].

When running samples from a reaction mixture of the chain terminator method, as in the experiments shown in fig.2, 1 μl sample was heated at 90°C for 3 min in 4 μl formamide/dye mixture [5]. This was loaded onto the gel using a finely drawn glass capillary. Different amounts of samples were used in the experiments shown in fig.1.

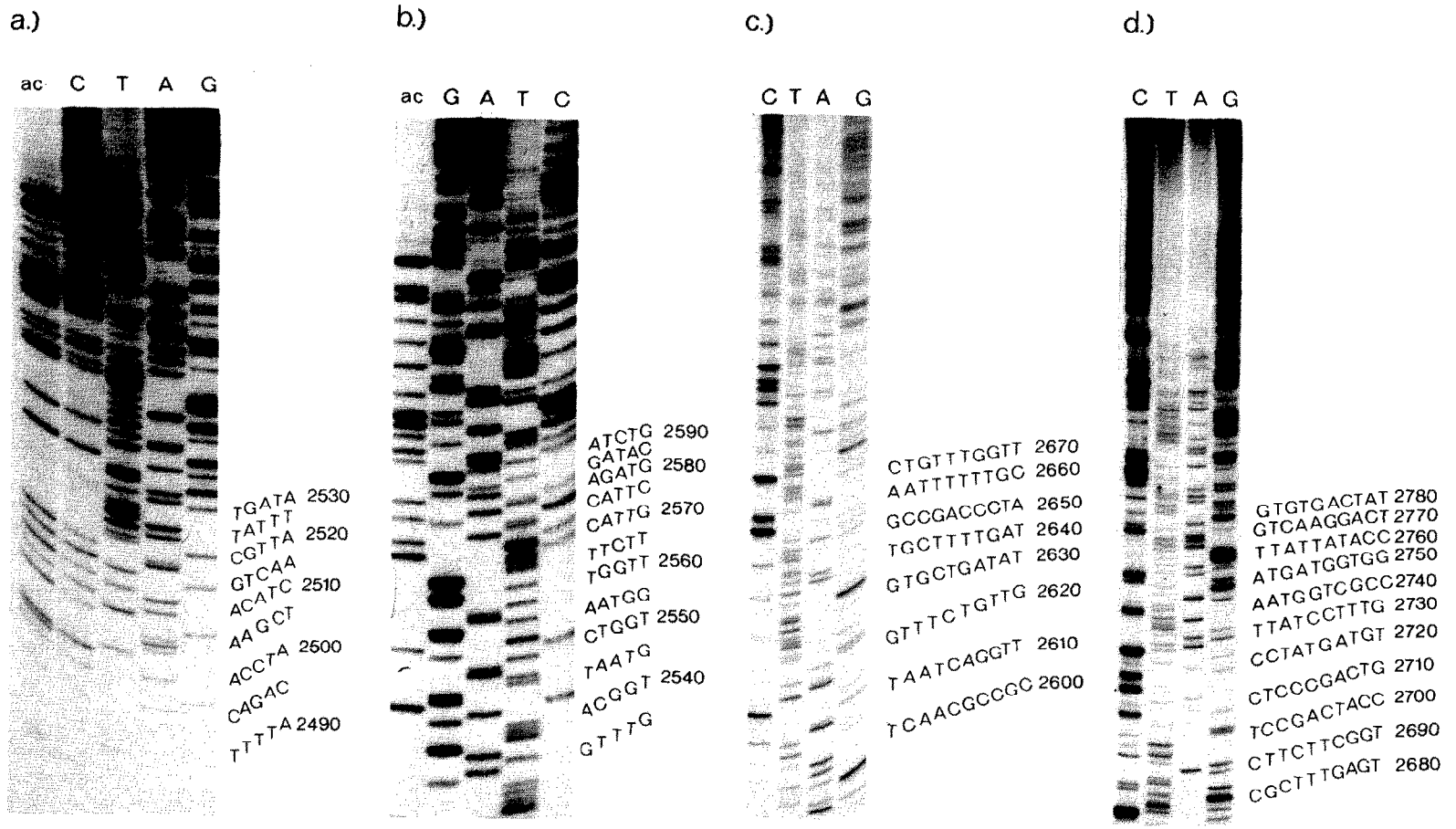

Fig.1. Autoradiographs of acrylamide gel electrophoreses from experiments using the terminator method [3] in which restriction enzyme fragment A16 [4] was used as primer on the complementary strand of ϕX174. Conditions were as in [3]. Incubations were carried out in the presence of the following concentrations of terminators: aC, 5 mM AraCTP; C, 0.1 mM ddCTP; T, 0.2 mM ddTTP; A, 0.1 mM ddATP; G, 0.2 mM ddGTP. All gels were 8% acrylamide. The top 5–9 cm of the gels are not included in the figure. Other conditions are: (a) Gel 0.4 mm thick, slots 14 mm wide. Electrophoresis for 2 h at 25 mA. (b) Gel 1.5 mm thick, slots 14 mm wide. Electrophoresis for 8 h at 45 mA. (c) Gel 0.6 mm thick, slots 8 mm wide. Electrophoresis for 6 h at 30 mA. (d) Gel 0.6 mm thick, slots 8 mm wide. Electrophoresis for approx. 10 h at 30 mA. The deduced sequences are those of the viral strand of ϕX and are written from left to right and upwards so as to come opposite the part of the gel to which they refer. The numbering is from the revised sequence (to be published) and is slightly different from that used in [6].

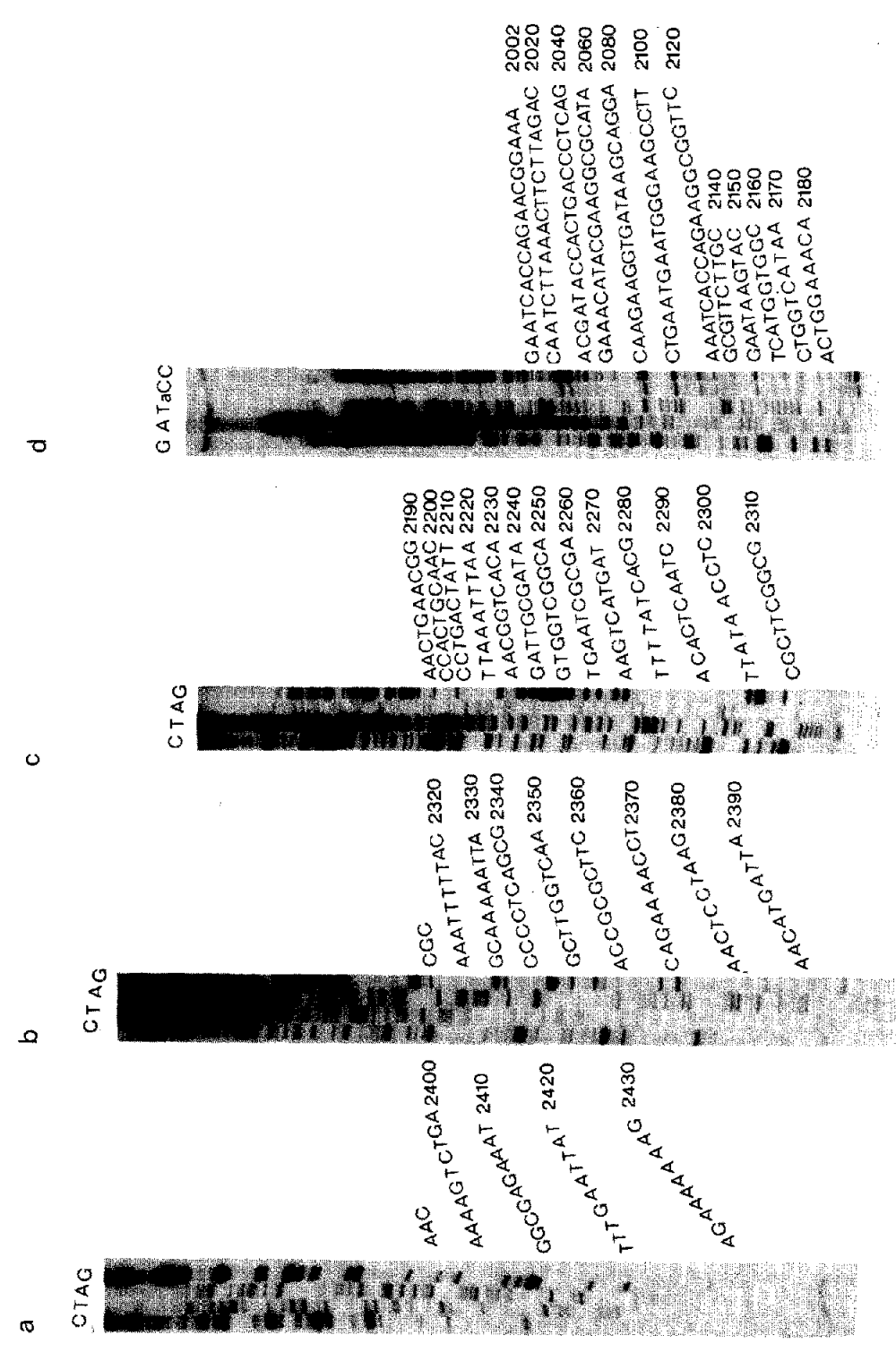

Fig.2. Autoradiographs from experiments, as in fig.1 but priming with fragment A16 on the viral strand of φX. All gels were 0.4 mm thick and were run at 25 mA. Slots were 5 mm wide. Concentrations of terminators were: (a) C, 0.1 mM ddCTP; T, 0.2 mM ddTTP; A, 0.1 mM ddATP; G, 0.2 mM ddGTP. (b) C, 0.1 mM ddCTP; T, 0.1 mM ddTTP; A, 0.05 mM ddATP; G, 0.2 mM ddGTP. (c), as (b) but 3 μCi [α-^{32}P]dATP (spec. act. 250 mCi/μmol) was present instead of the normal 1 μCi in the A incubation. (d) aC, 1.2 mM AraCTP; C, 0.05 mM ddCTP; T, 0.1 mM ddTTP; A, 0.05 mM ddATP and 3 μCi [α-^{32}P]dATP; G, 0.05 mM ddGTP. Other conditions were: (a) 8% acrylamide, 2 h electrophoresis. (b) 8% acrylamide, 3.5 h. (c) 8% acrylamide, 5 h. (d) 6% acrylamide, 6 h. The sequence deduced is that of the complementary strand of φX.

Gels were run at 25 mA (initially 1.5 kV, dropping to about 1.3 kV after heating up). Under these conditions, in an 8% gel, the bromophenol blue dye reached the bottom of the gel in about 1.5 h and the xylene cyanol FF (equivalent to an 80-base oligonucleotide) in about 3 h.

3. Results and discussion

Figure 1 shows autoradiographs of a number of gels using the termination method and priming with restriction enzyme fragment A16 [6] on the complementary strand of ϕX174. This fragment is 25 nucleotides long and was not split from the extended nucleotides after the DNA polymerase reactions. The various electrophoreses were run for different times and from the four gels it is possible to read off a consecutive sequence of 294 nucleotides. Figure 1b shows a gel of conventional thickness (1.5 mm). The gel in fig.1a was 0.4 mm thick but the loading wells were the same width as in fig.1b. The bands are clearly sharper on this thin gel. In the other two experiments (fig.1c,d) the gels were thin and the loading wells narrower. The sequence shown is in the coding region for the G gene of ϕX174 and essentially confirms the sequence reported [5,6] except for a change of an A to a T at position 2616 and a G to an A at position 2731.

Figure 2 shows the results of experiments in which the same restriction enzyme fragment (A16) has been used as primer on the viral strand of ϕX. All of these were run on thin gels and the sample wells were 5 mm wide. This width is quite adequate and makes it possible to run four to five experiments on one gel slab 20 cm wide. From the four gels a sequence of 437 nucleotides can be read. In fig.2d consecutive oligonucleotides of up to 465 residues long can be resolved – very much longer than is possible with thicker gels. Although individual bands in runs are actually overlapping one another in this region, it was possible to estimate the number of nucleotides in a run from the thickness of the bands and the first error in reading this gel was at positions 2001–2004, where A–A–A was estimated instead of the correct A–A–A–A. This was of course a particularly favourable case and it should not be assumed that an unknown sequence read out this far could be regarded as established from a single experiment. Beyond position 2000 there is still a pattern of bands from which an approximate sequence could be read and which could be used to establish overlaps with patterns from other priming experiments.

This sequence contains the complete untranslated region between the F and G genes, which was particularly difficult to work out with earlier methods [7], largely due to two symmetrical sequences which could form secondary structure loops during the electrophoresis. Using the thin gels at high temperatures these loops do not seem to form and it is possible to read the sequence correctly from the autoradiographs shown in fig.1. It is also evident that a C–G sequence had been missed out in the original sequence (positions 2310–2311 in fig.1, or position 2303 in [6]). The most extensive error in the provisional sequence of ϕX [6] was at positions 2217–2228, where fig.1 of this paper shows that six nucleotides have been missed out. The revised sequence has been confirmed in other experiments with fragment A16 and by priming with fragment Q3c on the complementary strand. Other revisions of the ϕX sequence will be discussed in a subsequent publication.

References

[1] Sanger, F. and Coulson, A. R. (1975) J. Mol. Biol. 94, 441–448.
[2] Maxam, A. M. and Gilbert, W. (1977) Proc. Natl. Acad. Sci. USA 74, 560–564.
[3] Sanger, F., Nicklen, S. and Coulson, A. R. (1978) Proc. Natl. Acad. Sci. USA in press.
[4] Reid, M. S. and Bieleski, R. L. (1968) Anal. Biochem. 22, 374–381.
[5] Air, G. M., Sanger, F. and Coulson, A. R. (1976) J. Mol. Biol. 108, 519–533.
[6] Sanger, F., Air, G. M., Barrell, B. G., Brown, N. L., Coulson, A. R., Fiddes, J. C., Hutchison, C. A., Slocombe, P. M. and Smith, M. (1977) Nature 265, 687–695.
[7] Fiddes, J. C. (1976) J. Mol. Biol. 107, 1–24.