FLUOROGRAPHIC DETECTION OF NUCLEIC ACIDS LABELLED WITH WEAK β-EMITTERS IN GELS CONTAINING HIGH ACRYLAMIDE CONCENTRATIONS

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1. Introduction

The separation of tRNAs by polyacrylamide gels has been restricted to the use of either unlabelled material detected by dyes such as ethidium bromide, or of ^{32}P-labelled samples. Likewise, sequencing by polyacrylamide gels of radioactive nucleic acids [1,2] has involved essentially ^{32}P-labelled material. This situation stems from the fact that ^{32}P can be detected without prior drying of the gel, whereas with isotopes of weak β-emission energy such as ^{3}H, ^{14}C and ^{35}S, drying of the gel is mandatory. However because gels used for nucleic acid analyses contain low acrylamide to bis-acrylamide ratios (≤30) and high (>10%) acrylamide concentrations, they inevitably crack during the drying process whether or not treatment with dimethylsulfoxide (DMSO), 2,5-diphenyloxazone (PPO) and water according to [3] has been performed. Consequently, localization on the latter gels of spots corresponding to nucleic acids labelled with isotopes of weak β-emission energy has required scintillation counting of individual gel slices, a tedious procedure with low resolution. Since ^{3}H and ^{14}C are routinely used in many laboratories for a wide range of experiments, a technique that could obviate slicing of the gels would greatly facilitate studies of nucleic acids.

In this paper we describe a simple method for drying various types of polyacrylamide gels routinely used in nucleic acid research.

2. Materials and methods

[^{3}H]- and [^{14}C]valine (28 Ci/mmol and 250 mCi/mmol, respectively) were from the CEA, Saclay, and [^{35}S]methionine (550 Ci/mmol) and [^{3}H]ATP (30 Ci/mmol) from New England Nuclear. *Escherichia coli* and yeast tRNAs were from Sigma. The gel slab dryer was either the Bio-Rad model 224 or an apparatus constructed in the laboratory workshop; 3.2 mm-thick porous polyethylene plates were used. Autoradiography was performed with Kodak X-Omat R films. Conditions for adenylation, or aminoacylation were as in [4,5]. Highly purified *E. coli* tRNA nucleotidyltransferase was a kind gift of D. Carré.

Polyacrylamide slab gels (20 × 16 × 0.2 cm) containing 12% acrylamide, 0.6% bis-acrylamide and 7 M urea were prepared as in [6], those containing 20% or 25% acrylamide, and 0.67% or 0.83% bis-acrylamide, respectively, and 7 M urea, as indicated in [7], and the two-dimensional gels (10% acrylamide and 0.5% bis-acrylamide, followed by 20% acrylamide and 1% bis-acrylamide) according to [8], except that unless indicated otherwise, running buffers (constantly recycled), sample buffers and gels were at pH 7, and electrophoresis was carried out at 4°C as in [9]. Following at least a 2 h pre-run at 800 V, the samples were layered onto the gel and electrophoresis performed at the same voltage. After the times indicated, the gel was treated with 10 vol. 10% cold trichloroacetic acid for 30 min at room temperature, then with DMSO, PPO and water as in [3]; where indicated it was soaked for 20 min in 20 vol. 70% methanol at room temperature, and dried at ~70°C. The gel was finally exposed at −70°C with a flash-activated film [10], and the fluorogram obtained was scanned using a Joyce-Loebl microdensitometer.

3. Results and discussion

Polyacrylamide slab gels are commonly dried by

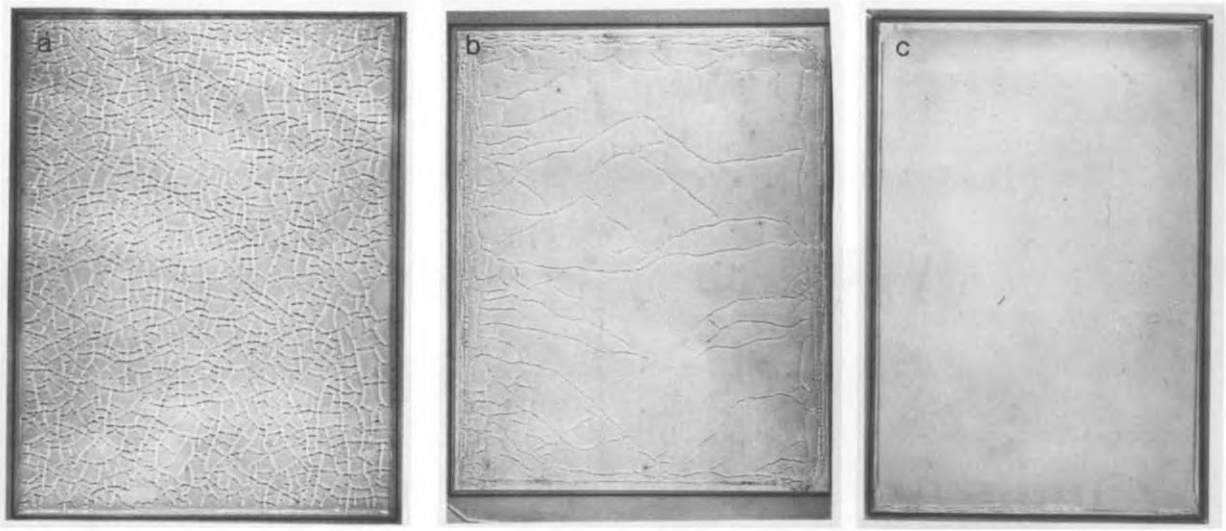

Fig.1. Polyacrylamide gels dried under different conditions. Polyacrylamide gels (20%) containing 0.67% bis-acrylamide and 7 M urea [7] were prepared as in section 2. After the 10% cold trichloroacetic acid and DMSO–PPO–water treatment, the gels were further soaked where indicated (b,c) in methanol before drying. They were then dried without (a,b) or with (c) a (second) porous polyethylene plate (fig.2).

applying heat on one side and vacuum on the same or the other side of the gel, a metal screen or a porous polyethylene plate (placed on the side of the gel to which vacuum is applied) allowing uniform dessication of the gel. However, gels containing low acrylamide to bis-acrylamide ratios (≤ 30) and >10% acrylamide crack upon drying (fig.1a). If such gels are soaked for 20 min in 70% methanol (resulting in a 15–20% decrease of the gel surface) following the DMSO–PPO–water treatment, they are less prone to cracking upon drying than without methanol treatment (fig.1b). Cracking occurs because the surface of these gels becomes vitrified upon drying so that water

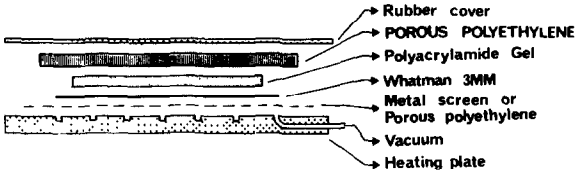

Fig.2. Schematic cross-section of drying apparatus. The porous polyethylene plate placed above the gel corresponds to the modification of the earlier apparatus.

droplets remain trapped on the side of the gel opposite the vacuum; only after cracking of the gel and evaporation of the droplets can the gel ultimately dry. The problem of gel cracking was circumvented by applying

Table 1
Behaviour of gels in different drying conditions

Acrylamide (%)	Bis-acrylamide (%)	{Bis-acrylamide}² × {Acrylamide}	Without porous plate	With porous plate		Gel prep. [ref.]
			Without or with methanol	Without methanol	With methanol	
12	0.6	4.3	–	+	+	[6]
20	0.67	9.0	–	+	+	[7]
25	0.83	17	–	+	+	[7]
20	1	20	–	–	+	[8]

–, gel cracked; +, gel dried without cracking

Gels were prepared as in section 2. After the DMSO–PPO–water treatment, they were soaked where indicated in methanol (section 2) and dried without or with a (second) porous polyethylene plate (fig.2).

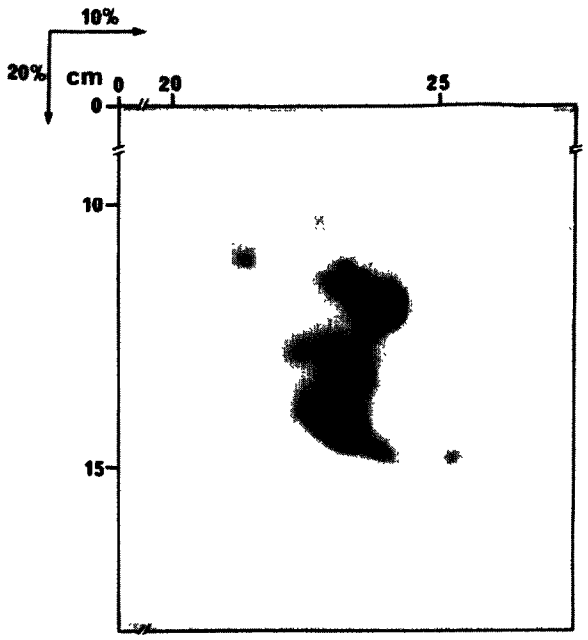

Fig.3. Fluorogram of yeast [^{3}H]A-tRNAs separated by two dimensional polyacrylamide gel electrophoresis. [^{3}H]A-tRNAs (2 μg) (50 000 cpm) were used for two dimensional (10% and 20% polyacrylamide) gel electrophoresis in the conditions of [8] and in section 2, except that in the first dimension the gel (40 × 16 × 0.15 cm) was run at 450 V for 20 h and in the second at 450 V for 40 h. After electrophoresis the gel was treated as in section 2, soaked in methanol and dried as shown in fig.2. The gel was then exposed at −70°C using a flash-activated film; the fluorogram obtained after 10 days exposure is presented.

the vacuum simultaneously to both sides of the gel, that is, by sandwiching the gel between a porous polyethylene plate and the metal screen (fig.2), or between two porous polyethylene plates (instead of one plate) in the case of the apparatus constructed in the laboratory workshop (not shown). Since vacuum is now applied to the both sides of the gel, the heating plate and the vacuum source can be on the same side of the gel (fig.2) or on opposite sides, with identical results. Under these conditions the gel remains intact upon drying (fig.1c; see also table 1).

Table 2
Stability of [^{3}H]Val-tRNA during electrophoresis

pH	Temp.	Electrophoresis (h)	Peak surface (%)
		3.5	100
7	4°C	5	95
		8.5	80
8.3	30–40°C	3.5	25

[^{3}H]Val-tRNA (15 000 cpm) was electrophoresed on a 12% polyacrylamide gel [6] as in section 2, for the times indicated in the table at 4°C and pH 7 [9], or at room temperature (30–40°C inside the gel) and pH 8.3 [6]. The gel was then fluorographed, treated with methanol and the fluorogram scanned (section 2). The peak surfaces were calculated; the 3.5 h value (4°C and pH 7) is taken as 100%

Four types of polyacrylamide gels routinely used for the analysis and sequencing of ^{32}P-labelled nucleic acids [6–8] were used to follow the improvement brought about by soaking the gel in methanol prior to drying, and by including a (second) porous plate (indicated in capital letters in fig.2) during the drying process. Table 1 shows that the 4 types of gels examined cracked if the (second) porous plate was omitted. The ratio of acrylamide to bis-acrylamide is not the only factor responsible for gel cracking, since gels containing low acrylamide to bis-acrylamide ratios (~30) but only 10% polyacrylamide [7] dried without deleterious effect even without methanol treatment or without a (second) porous plate (not shown). Therefore cracking is due to a combination of the low acrylamide to bis-acrylamide ratios and high polyacrylamide concentrations (>10%).

To predict the possible difficulties that might arise during drying if the (second) porous plate is omitted, a calculation based on {bis-acrylamide}^{2} × {acrylamide} was found convenient. Cracking can be expected for values ≥4.3 (table 1); it becomes more extensive as this value increases. However when a (second) porous plate is included, all gels dry correctly except for the 20% acrylamide, 1% bis-acrylamide gels ({bis-acrylamide}^{2} × {acrylamide} = 20) which crack if the methanol treatment is not performed. Furthermore, the edges of the gels soaked in methanol are perfectly intact whereas those of gels dried without this treatment present minor cracks.

Even though methanol efficiently helped to abolish cracking of the gels, it was important to define whether it decreases the fluorographic efficiency. We therefore examined the fluorographic efficiency of 12% or 20% polyacrylamide sequencing gels [6,7] soaked in methanol, using various β-emitters ([^{3}H]Val-tRNA, [^{14}C]Val-tRNA and [^{35}S]Met-tRNA). The fluorograms obtained were scanned and the surfaces of the peaks for each label were determined (not shown). In both kinds of gels and for all 3 labels, the efficiency is as good, whether or not the gels were soaked in methanol before drying. Thus methanol has the advan-

tage of preventing cracking of the gel without decreasing fluorographic efficiency. Therefore after the DMSO–PPO–water treatment, it is advisable to soak the gel in 70% methanol for 20 min at room temperature before placing it with its Whatman support between the porous plate and the metal screen and drying as usual.

An application of this technique (the separation of ^{3}H-labelled tRNAs on two dimensional polyacrylamide gels) is demonstrated in fig.3. Yeast tRNAs were adenylated in vitro using [^{3}H]ATP and tRNA nucleotidyltransferase [4]. They were then separated on two dimensional (10% and 20%) polyacrylamide gels [8]; the resulting fluorogram shows that the [^{3}H]A-tRNAs are separated into ~15 spots after short exposure times (fig.3) and into >30 spots after prolonged exposure (not shown).

For the separation of ^{3}H-, ^{14}C or ^{35}S-labelled aminoacyl-tRNAs the problem of deacylation during the electrophoretic runs commonly performed at pH 8.3 and room temperature must be taken into account. With the aim of diminishing the risk of hydrolysis of the ester linkage, we have routinely performed electrophoresis at pH 7 and 4°C [9]. Using a 12% polyacrylamide gel [6] we have compared the stability of [^{3}H]Val-tRNA at pH 7 and 8.3. Table 2 presents the decrease in Val-tRNA as a function of the duration of electrophoresis. Only 20% of the aminoacyl-tRNA is deacylated after an 8.5 h run at pH 7 and 4°C, as compared to 75% after a 3.5 h run in the conditions generally used (pH 8.3, room temperature). Thus the methodology described here can be directly applied to the analysis by one or two dimensional gels of aminoacyl-tRNAs with fairly stable ester linkages (such as Val-tRNAs). The ester linkage can further be stabilized 3–4-fold by blocking the NH_2 group of the amino acid [11].

A limitation of this method arises in the sequencing of nucleic acids: only fragments of $\gtrsim 15$ nucleotides can be visualized, since shorter fragments are eluted from the gel during the DMSO–PPO–water treatment. Consequently, the sequence of short oligonucleotides must be determined using other techniques.

In conclusion, this simple drying method extends the use of ^{3}H-, ^{14}C- and ^{35}S-labelling to nucleic acid analysis in sequencing gels [1,2,6,7] or in two dimensional polyacrylamide gels [8]. It now becomes possible to perform experiments such as to:

(i) Isolate ^{3}H, ^{14}C- or ^{35}S-labelled aminoacyl-tRNAs;
(ii) Sequence an isoacceptor tRNA (even in the presence of other tRNAs) charged with its cognate radioactive amino acid;
(iii) Isolate or sequence an mRNA labelled on its cap structure;
(iv) Analyze modified bases in DNA or RNA molecules.

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