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Note

Improved staining procedure for nucleic acids in polyacrylamide gels after complexing with nitroso compounds

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Dyes such as methylene blue, acridine orange, ethidium bromide and pyronine Y are used for the detection of unlabelled nucleic acids in polyacrylamide gels[1–5]. Usually the only method applied for immobilizing the nucleic acids is to insert the gels in acid in order to convert the salts into the free acids. Many species of nucleic acids, especially those having a low molecular weight, are fairly soluble in water or dilute acids, so that the stained bands of nucleic acids fade soon after the necessary destaining procedure has been completed. This paper describes an alternative fixation procedure that allows more stable staining of nucleic acids to be achieved.

EXPERIMENTAL

Ribonucleic acids were prepared from *Saccharomyces cerevisiae* according to Holley[6]. Crude extracts were purified over DEAE-Sepharose CL-6B (Pharmacia, Uppsala, Sweden). The elution buffer system was a gradient of Tris–chloride (0.15–1.0 *M*). Fractions containing ribonucleic acids were pooled, ice-cold ethanol (96%) was added and the precipitate was freeze-dried. The lyophilized samples of ribonucleic acids were used for polyacrylamide gel electrophoresis (PAGE). Ribonucleic acid purchased from Serva (Heidelberg, F.R.G.) could not be used because of their low molecular weight, all molecules migrating with the front.

PAGE was carried out as described earlier[7]. In a block gel apparatus, a separation gel with 20% acrylamide (Merck, Darmstadt, F.R.G.), cross-linked 1:30 with bismethyleneacrylamide (Bis) (Merck), pH 7.5, was used. This gel is very suitable for nucleic acids of low molecular weight. The electrophoresis buffer was a Tris–glycine system[7]. For a run of 4 h a voltage of 300 V and a starting current of 100 mA were used. The apparatus was cooled with running water.

Samples of lyophilized nucleic acids were dissolved in an aqueous solution of acrylamide, Bis and Tris in the same proportions as used for preparing the separation gel.

On each lane $1 \cdot 10^{-4}$ g of ribonucleic acid were separated. To the samples of

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ribonucleic acids 8% of one of the following solutions were added for complexing: 0.1% Amido Black 10B (Merck) in methanol–glacial acetic acid–water (5:1:5); 1% 4-nitroso-N,N-dimethylaniline (Merck) in the same solvent; 1% disodium 2-hydroxy-1-nitrosonaphthalene-3,6-disulphonate (nitroso-R salt) (Merck) in the same solvent; or 1% 2-nitroso-1-naphthol (Merck) in aqueous solution.

The dyed samples and the control were separated by PAGE. The slab gels were stained with methylene blue[1] or acridine orange[3]. Running water was used for destaining in every instance. For documentation the gels were photographed.

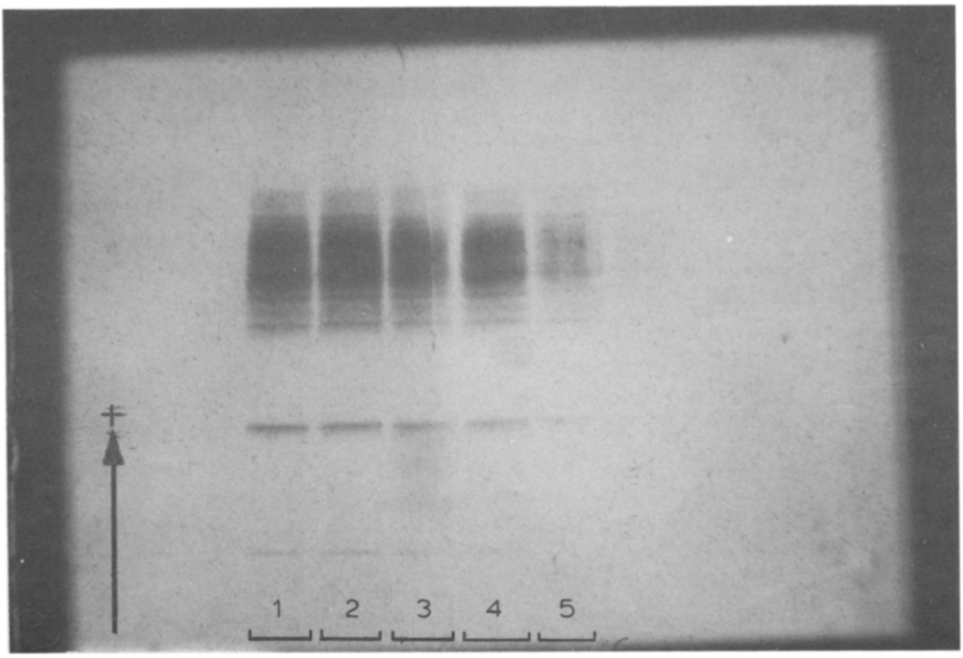

Fig. 1. Electropherogram of RNA from *Saccharomyces cerevisiae*. 1, RNA + Amido Black 10B; 2, RNA + nitroso-R-salt; 3, RNA + 2-nitroso-1-naphthol; 4, RNA + 4-nitroso-N,N-dimethylaniline; 5, RNA in aqueous solution. Staining was carried out with methylene blue and destaining with water. Each lane contains $1 \cdot 10^{-4}$ g of RNA.

RESULTS

Fig. 1 shows the staining results for yeast RNA separated on 20% polyacrylamide gel with methylene blue, destained in running water for 18 h. Easily the most intense dye–RNA interaction is obtained when the RNA had been pre-incubated with nitroso compounds. Further destaining results in the early disappearance of the RNA that had not been treated with the nitroso compounds before. No differences in staining intensity depending of the kind of nitroso compound used could be observed. Amido Black 10B, 4-nitroso-N,N-dimethylaniline, nitroso-R salt and 2-nitroso-1-naphthol gave essentially the same staining results. As these reagents had been dissolved in different solvents, the effect of the solvent seems unimportant.

Therefore, it seems that the presence of the nitroso group and its interaction with the nucleic acid is solely responsible for the improved staining.

Fig. 1 also shows that exactly the same electrophoretic bands appeared with or without addition of the nitroso compound. The interaction of the fixation agent therefore does not influence the electrophoretic mobility of the RNA.

Essentially the same staining results were obtained with acridine orange. We suggest that the staining of RNA in polyacrylamide gel is improved in general by the addition of organic compounds carrying a nitroso group.

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