Electrophoresis of nucleic acids in silica gel

The use of freshly prepared silica gel as an anticonvection medium in zone electrophoresis was reported orginally by CONSDEN, GORDON AND MARTIN[1] who used these gels in the electrophoretic fractionation of amino acids and peptides. GORDON *et al.*[2] observed that proteins would not migrate in silica gel, apparently because the pores of the gel were too small to permit movement of the large protein molecules. This laboratory has reported recently a method for the rapid preparation of silica gels containing appropriate electrophoretic buffers and their use in zone electrophoresis[3]. This note reports the results of some attempts to carry out electrophoretic fractionations of RNA and DNA by electrophoresis in silica gel.

Crude RNA from yeast was prepared by the method of CRESTFIELD, SMITH AND ALLEN[4] and was fractionated by the use of 1 *M* NaCl. Both the NaCl-soluble and -insoluble fractions were precipitated with cold ethanol and washed with 65 % ethanol until free of NaCl. They were then dissolved in water and adjusted to pH 7. DNA was prepared by the method of HURST[5] and was freed of a small amount of contaminating protein by shaking with chloroform and amyl alcohol[6]. The nucleic acids were subjected to electrophoresis in silica gel at pH 7.0 using triethanolamine buffer of 0.1 ionic strength. The silica gel was prepared as described previously[3]. The NaCl-soluble RNA moved rapidly as a narrow band in the silica medium. The NaCl-insoluble RNA, on the other hand, exhibited varying degrees of mobility in the gel. A portion of this fraction migrated as rapidly as the soluble RNA and the remainder showed lesser mobilities in varying degrees, the slowest material having about one-fourth the mobility of the fastest. The channels containing the two fractions were cut in 1-cm segments following electrophoresis, the RNA eluted with 0.1 *M* HCl, and the absorbancies read in the spectrophotometer at 260 mμ. The results are given in Fig. 1. Samples of the leading portion, center, and trailing portion of the NaCl-insoluble RNA were taken and resubmitted to electrophoresis in separate inlays, and it was found that each sample migrated as before. When the three samples were mixed and resubmitted to electrophoresis from a single inlay, three bands were obtained with mobilities similar to the starting materials.

DNA, in contrast to RNA, did not migrate in the gel but remained bound at the site of application. Experiments to determine how readily nucleic acids could

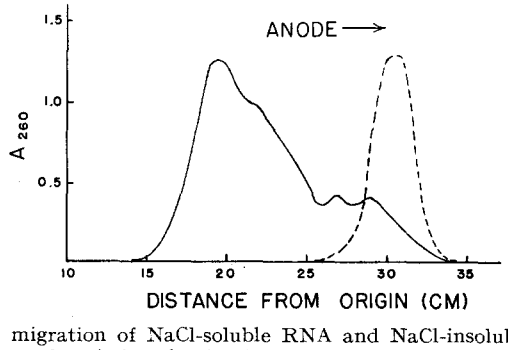

Fig. 1. Electrophoretic migration of NaCl-soluble RNA and NaCl-insoluble RNA in silica gel. Conditions: 15 V/cm for 4.3 h; triethanolamine (acetate) buffer, pH 7.0, *I* 0.1. – – –, NaCl-soluble RNA; ——, NaCl-insoluble RNA.

Abbreviations: RNA, ribonucleic acid; DNA, deoxyribonucleic acid.

be extracted from silica gel were carried out as follows: solutions of soluble and insoluble RNA, and DNA, in 0.1 *M* phosphate buffer at pH 7 were mixed separately with equal volumes of silica sol (6 % in SiO_2) and allowed to gel. Each gel was then homogenized with 5 vol. water in a Potter-Elvehjem glass homogenizer for 1 min. The gel residue was centrifuged down and the nucleic acid content in the supernatant determined. 98 % of the NaCl-soluble RNA, 60 % of the NaCl-insoluble RNA, and about 1 % of the DNA were extracted from the gel by this procedure. As shown in Table I, extremes of pH are required for extraction of DNA from the gel. At pH 1 destruction of the DNA occurs, while at pH 9.2 or 10.2 extraction of the DNA is probably facilitated by changes in the gel structure.

TABLE I

EXTRACTION OF DNA FROM SILICA GEL

Final concentration of DNA in the gel prior to extraction is 0.1 %. In all procedures the volume of extracting solution used is five times the volume of the undried gel.

Extraction procedure	*DNA in supernatant (% of total A_{260} units)*
Homogenization with water	0.5–2
Homogenization with 2 *M* NaCl	2
Gel dried *in vacuo*, 0°:	
first homogenization with water	1.8
second homogenization with water	0.5
third homogenization with water	0.2
Gel dried with acetone–ether, homogenized with water	0
Gel held at pH 10.2, 0.5 h, 20°	92
Gel held at pH 9.2, 0.5 h, 20°	53
Gel held at pH 1.0, 1 h, 100°	93

Freshly prepared silica gel may prove to be useful in the fractionation of RNA, separation of RNA from DNA and protein, or in other procedures involving the separation of compounds of low and high molecular weights. The ability of peptides and RNA to migrate electrophoretically in the gel while proteins and DNA remain bound at the origin suggests that the gel acts as a molecular sieve which slows or prevents the movement of large molecules while allowing small molecules to migrate without hindrance.

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