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Note

Polyacrylamide gel structure resolved?

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The appearance of scanning electron micrographs published in connection with a study of crystal growth[1] forms the basis of doubts recently cast[2] upon current interpretations of the structure and hence operation of polyacrylamide gels[3–5], so widely used for electrophoretic separation of proteins[3–6] and RNA[3,7–11] and for molecular sieve filtration of proteins[5].

The reliability of the scanning electron microscope (SEM) evidence[1] is of crucial importance, for the 2.5% gels that were examined exhibited a matrix enclosing pores 2–10 μm in diameter; if these micrographs portray the actual pore structure of gels used for electrophoretic studies, and if the pores are, as implied, parts of a fluid-filled continuum, then there is no reason why electrophoresis in acrylamide gels should be appreciably different from electrophoresis in free solution. Yet theoretical analyses of rates of protein migration in gels suggest the existence of pore sizes in the range of protein molecular dimensions[4–6]. The 2.5% polyacrylamide concentration used in making the micrographs[1] was about that used for high-molecular-weight RNA fractionation (but not proteins) by electrophoresis[3,6–11]. At the same low acrylamide concentration various parameters, such as running temperature and voltage gradient, differently affect the mobilities of single and double-stranded RNA[10]. The temperature of polyacrylamide polymerization also affects mobilities of various RNA species, presumably by affecting pore stability or shape. The molecular weight of a putative ribosomal RNA precursor can be estimated to be 1.8–2.5 $\times$ 10^6 depending upon the polymerizing temperature of the gel[11].

DNA renatures more rapidly in 2.4% polyacrylamide gels than in free solution following electrophoresis, suggesting that the gel structure confines free movement[12]. Confining structures of protein-containing gels were demonstrated by Berezin *et al.*[13]. If a 2.5% polyacrylamide gel, even supposing an enormous amount of bound water, is to have a confining structure, this small amount of polymer must be more evenly distributed than in the large sheets seen in the micrographs[1]. Clearly, there is weighty evidence against the existence of pores as large as those indicated by the scanning electron micrographs. The gel structure is usually theoretically envisaged as a mesh of linear polyacrylamide cross-linked with N,N′-methylenebis(acrylamide)[2–6].

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It is well known that the rate of freezing of hydrated specimens affects ice crystal size[14]. The published micrographs were prepared by freezing on the cold table of a freeze-drying unit[1]; this would produce a relatively slow freezing rate and, presumably, large ice crystals[14].

EXPERIMENTAL

2.5% acrylamide 0.125% N,N'-methylenebis(acrylamide) gels were prepared with recrystallized monomers according to Loening[7,8].

Pieces were excised from such gels and were first treated as outlined in Fig. 1. The pieces for critical-point drying were dehydrated by passing them through a series of ethanol–water (or acetone–water) mixtures of increasing ethanol (acetone) concen-

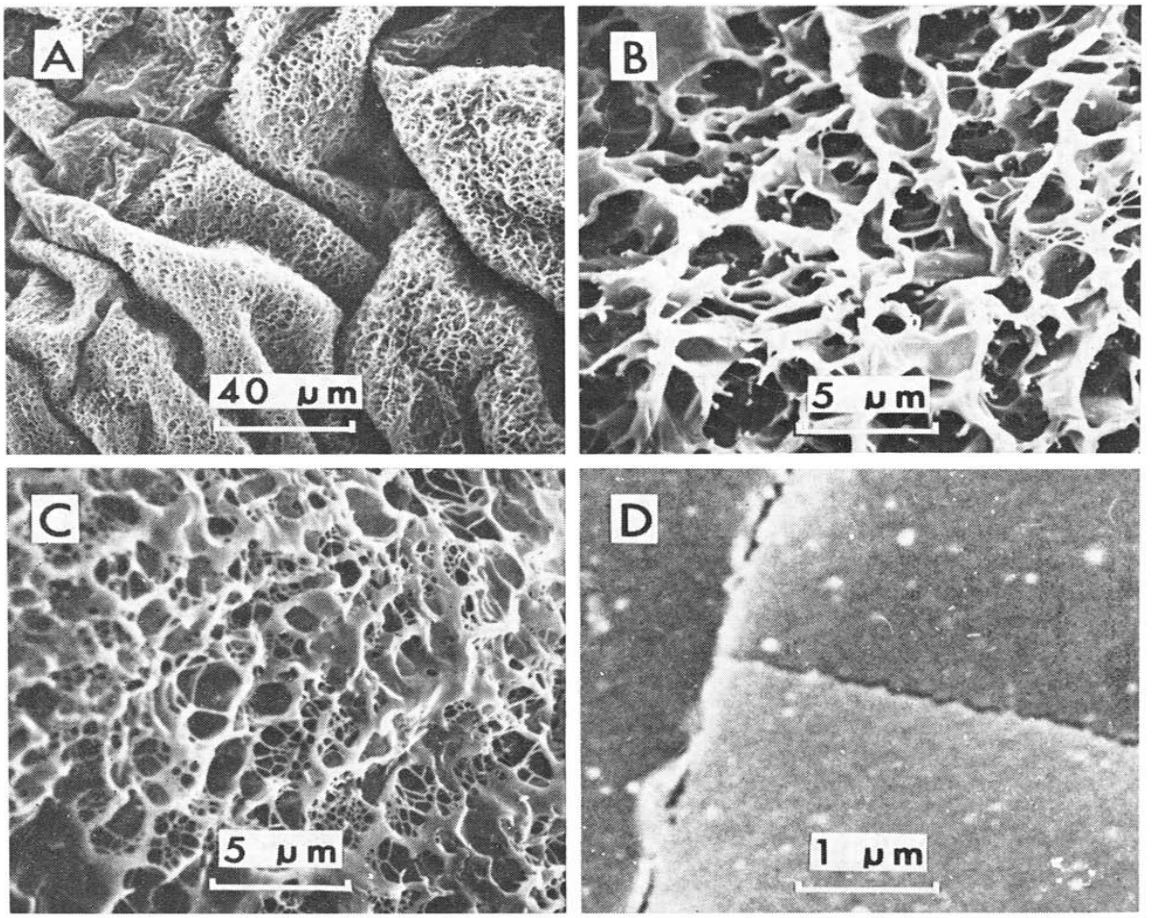

Fig. 1. The effect of preparation techniques for scanning electron microscopy on the visualized structure of polyacrylamide gels. A and B, 2.5% acrylamide gel pieces were freeze-dried essentially according to Blank and Reimschuessel[1]. C, A similar gel piece frozen in liquid nitrogen slush at −210° and then freeze-dried. D, Gel pieces were critical-point dried from ethanol (see Experimental). A fissure appearing in the critical-point dried piece (D), (probably caused by the initial cutting) as well as dust particles, were used to obtain a focus of this seemingly homogeneous piece.

tration. From 100% ethanol (or acetone) the gel pieces were transferred to a critical-point drying apparatus (Polaron) and dried from liquid carbon dioxide. Specimens were coated with carbon and gold–palladium before viewing in an HHS-2R scanning electron microscope (Hitachi).

RESULTS AND DISCUSSION

To try to establish the cause of the apparent paradox[2] between the theory which is supported by the separation data[3–5] and the structure visible by microscopy[1], we decided to study further the structure of polyacrylamide gels by scanning electron microscopy. We prepared the gels for scanning by various methods to examine whether ice crystal formation during preparation could distort the internal structure. Micrographs prepared by freeze drying are shown in Figs. 1A and B. The structure appears similar to that reported[1], confirming those results. Gels frozen at faster rates (in liquid nitrogen at −210°) had smaller pore sizes (Fig. 1C). Gels were also critical-point dried from ethanol or acetone. This technique, while introducing about 50% shrinkage from the acetone or ethanol treatment, is unlikely to bring about a change in structure that would diminish pore size (apart from the shrinkage), or reduce fibre diameter[1,4].

Even at high magnifications, no pores, fibres, or other internal structures are apparent (Fig. 1D). Allowing for shrinkage from the hydrated state, this would suggest a pore size of less than 20 nm. The maximum resolution of the SEM used is 10 nm. From calculations of pore size, the thickness of the polyacrylamide fibre has been estimated as 0.5–0.8 nm[4,5]. If the acrylamide were in single- or even in 15-fibre bundles it could not be resolved with the techniques we used. Thus, it seems that the previously reported large pore structure[1] may be interpreted as being caused by ice crystals formed during freezing which push the polyacrylamide mesh into an array appearing sheet-like after the water is sublimed. This large pore formation during freezing would explain why it is simpler to elute compounds from gels following freezing.

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