Microchromatographic separation of ribonucleic acids from proteins on nitrocellulose membranes

Synpor nitrocellulose membrane filters (Chemapol, Prague) were recently shown to be a suitable medium for the chromatographic deproteinization[1] of very small amounts of biological fluids as well as for a simple and rapid quantitative determination of proteins by circular chromatography[2]. The microtechniques mentioned above were based on the fact that most proteins were strongly adsorbed to the nitrocellulose membranes especially from acid aqueous solutions (*e.g.* pH 3.7), whereas a series of low-molecular substances migrated freely from the start during chromatography. In other experiments[3] devoted to the investigation of the chromatographic behaviour of some nucleic acids it was found that even high molecular weight ribonucleic acids (RNA) (but not DNA) migrated with the front of neutral or slightly acid buffers on nitrocellulose strips, as could be expected from the results of filtration experiments of other authors[4,5].

In the present work we attempted to find suitable conditions for a simple and gentle microchromatographic separation of RNA and proteins in the neutral pH region, using nitrocellulose membranes and avoiding the use of organic solvents (phenol, chloroform) commonly used for similar purposes[6].

Strips 1 × 2 cm or 2 × 3 cm of nitrocellulose membranes: Synpor 6,8,9,10 (Chemapol, Prague) and MF 10 (Membranfiltergesellschaft, Göttingen) were used for chromatography performed in a way similar to that described previously[1]. Before use, the membranes had to be washed in boiling distilled water to remove the remnants of organic impurities and thus guaranteeing more regular separations and better staining of the spots. A Tris–borate[7] (pH 7.5) and a citrate[5] (pH 7.0) buffer were used as developing solutions.

Rabbit serum and a mixture of soluble ribonucleic acids (RNA) isolated from chicken leukaemic myeloblasts (a gift from Drs. ŘÍMAN and TRÁVNÍČEK, Czechoslovak Academy of Sciences, Prague) served as model substances for chromatography. Orientation experiments were also done with fresh homogenates of rat livers, kidneys, lungs and spleen. The samples having a concentration of about 1–5 % of proteins and RNA, respectively, were applied to the membranes in volumes of the order 0.1–0.01 μl. Chromatography usually lasted 30–40 min at laboratory temperature.

The strips were first stained (after drying at 80° for 5–10 min) with 0.01 % nigrosine in 3 % trichloroacetic acid to detect the protein spots. The strips were then washed in water and subsequently stained for RNA with 0.5 % toluidine blue in 10 % aqueous ethanol or with 3 % methyl green in water. When differential staining was not necessary, the chromatograms could be detected directly with toluidine blue or methyl green. However, with these stains, the background of the chromatograms remained partly coloured even after exhaustive washing.

As can be seen in Fig. 1 a distinct separation of the serum proteins and of RNA was achieved at pH 7.5 especially on MF 10 membranes. Similar results were also achieved with Synpor 9 membranes, but the separation was usually less distinct at that pH, because both RNA and proteins formed rather diffuse and prolonged spots with tailing.

The protein spots had very sharp contours even at pH 7.5 on MF 10, similar to those on Synpor 6,8 or 9 membranes at pH 3.7, in addition their areas were pro-

portional to the protein concentration. Thus, the MF 10 membranes seem to be very convenient not only for separating RNA and proteins but also for the quantitative determination of protein concentration by circular chromatography[2] even in the neutral pH region. Synpor membranes were less suitable for this purpose at neutral pH's, since a part of the native serum proteins (mainly albumin) tailed and the

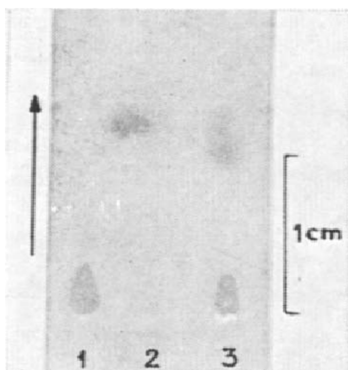

Fig. 1. Microchromatographic separation of RNA and serum proteins. 1 = Rabbit serum; 2 = RNA from chicken leukaemic myeloblasts (see text); 3 = mixture of samples 1 and 2. Nitrocellulose membrane MF 10, Tris–borate buffer, pH 7.5, chromatographed for 30 min, stained with nigrosine and toluidine blue.

contours of the spots were not sharp enough to enable exact protein determinations.

The results of membrane chromatography of the homogenates on MF 10 strips were similar to those of the model substances (*cf.* Fig. 1) and indicated the possibility of a simple and rapid single-step separation of free soluble RNA from the proteinous constituents of the homogenates, including both insoluble debris of the tissue and the soluble proteins.

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