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Note

Simple inexpensive apparatus for the rapid DEAE-cellulose chromatography of small quantities of glycoproteins

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Previously [1,2] we obtained apparently homogenous epithelial glycoproteins from extracts of fresh, human or rat, colonic epithelial cells by fractionation with a combination of agarose (A15 M) gel and DEAE-cellulose chromatography. These conventional methods were, however, unsatisfactory for the fractionation of small quantities of glycoproteins such as those we have isolated recently [3] from formalin fixed tissues. In this paper we describe an apparatus designed for the fractionation of small quantities of acidic glycoproteins by stepwise, centrifugal elution from micro-columns of DEAE-cellulose. The apparatus was constructed from commercially available inexpensive materials which can be used in a standard benchtop centrifuge. It was based upon a design used previously for the desalting of small volumes of solutions [4] and for micro step exclusion chromatography [5]. The procedure, therefore, provides the DEAE-cellulose counterpart of such gel chromatographic systems.

MATERIALS AND METHODS

Glycoproteins were obtained, as previously described, from fresh rat colonic epithelial cells [1,2] and from formalin fixed specimens of normal and diseased human large and small intestine.

Analytical techniques

Ketosidically linked sialic acids were measured, after saponification of the glycoprotein, with a miniaturized version of the procedure of Culling et al. [6]. Cellulose acetate electrophoresis was performed for 35 min at 300 V on strips (6 × 1 in.) of Sephraphore III (Gelman, Ann Arbor, Mich., U.S.A.) using Tris—barbital—sodium barbital buffer (pH 8.8, i=0.05) and a current of 1.5—2.5 per strip. Electropherograms were stained with alcian blue [7] and were scanned at 605 nm in the gel scanner attachment of a Beckmann 25 spectrophotometer.

Construction of micro column assembly

The apparatus, shown in Fig. 1, was constructed from a Gilson, P1000, pipette tip (7.2 × 0.95 cm) suspended in a 7.0 × 1.2 cm Falcon plastic test tube by means of a sleeve consisting of the upper 2.5 cm of a conical 1.5-ml Eppendorf centrifuge tube (O.D. top 1 cm). A glass wool plug was inserted into the constricted end of the pipette tip and the column of DEAE-cellulose (Whatman DE 22-fibrous) was packed as a slurry in 0.02 *M* pyridine hydrochloride pH 5.5. The DEAE-cellulose had an initial height of 3.0—3.2 cm and, after centrifugation at 1000 *g* for 5 min, a packed volume of 800 μl.

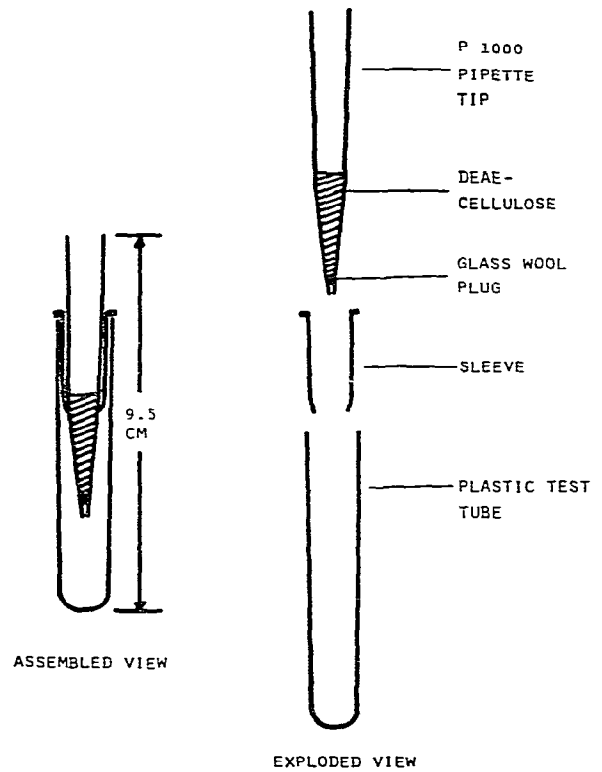

Fig. 1. Diagrams of exploded and assembled views of the apparatus for micro DEAE-cellulose chromatography.

Fractionation procedure

Glycoprotein extracts (100—200 μl containing up to 540 μg sialic acid) were mixed with an equal volume of 0.04 *M* pyridine hydrochloride and were then added to the column. The column was eluted with an aliquot (500 μl) of 0.02 *M* pyridine hydrochloride buffer pH 5.5 followed by aliquots (500 μl) of the buffer containing increasing concentrations of sodium chloride. Elution was carried out by centrifugation at 1000 *g* for 5 min. Following fractionation the column was regenerated by washing three times with aliquots (500 μl) of pyridine hydrochloride.

RESULTS

In previous studies [1,2] we were able to separate mixtures containing rat colonic epithelial glycoprotein and DNA by gradient elution, from conventional DEAE-cellulose columns with sodium chloride in 0.02 *M* pyridine hydrochloride pH 5.5. Since these columns could not be used with small quantities, preliminary studies were carried out with micro-columns of DEAE-cellulose. These demonstrated that such quantities could be separated by consecutive stepwise elution with 0.02 *M* pyridine hydrochloride pH 5.5 containing sodium chloride, the concentration of the sodium chloride being increased by 0.1 *M* between each elution. The micro-columns were then used to fractionate extracts of formalin fixed human colonic tissues. The columns were eluted with 500-μl aliquots of 0.02 *M* pyridine hydrochloride buffer pH 5.5 (once) and buffer containing the following concentrations of sodium chloride: (a) 0.02 *M* (twice); (b) 0.3 *M* (three times); (c) 0.35 *M* (once); (d) 0.5 *M* (twice) and (e) 2.0 *M* (twice). The eluents were combined to yield five fractions: 1 consisting of the buffer alone plus the 0.2 *M* eluent and fractions 2—5 consisting of the 0.3 *M*, 0.35 *M*, 0.5 *M* and 2.0 *M* eluents, respectively. Fig. 2 shows the

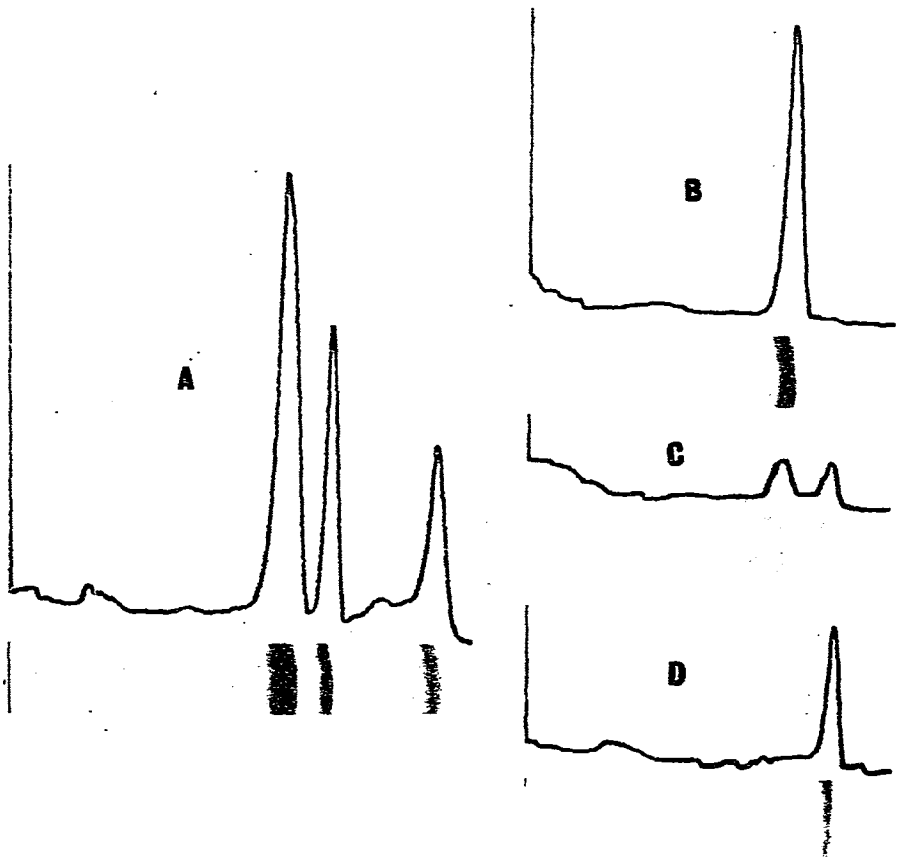

Fig. 2. Electropherograms and scans obtained from a representative fractionation. A = Unfractionated extract; B = fraction 2; C = fraction 3; D = fraction 4.

electropherograms and scans of fractions obtained from the DEAE-cellulose chromatography of a representative glycoprotein extract. Fractions 2 and 4 contained a single, apparently homogenous component while fraction 3 contained trace quantities of fractions 2 and 4. Typically fraction 5 (not shown in Fig. 2) contained one or two components with mobilities similar to DNA. Subsequent analysis showed that fraction 2 was essentially the only sialic acid containing component. The identity of the other fractions has not been established but presumably they contain one or more proteoglycans and possibly DNA.

This fractionation technique has been successfully applied to extracts of histologically normal resection margins and tumours from cases of carcinoma of the colon, specimens of colon from cases of ulcerative colitis and Crohn's disease and specimens of normal small intestine and small intestine from cases of Crohn's disease. It should be noted that, on occasion, sialoglycoprotein was eluted with 0.2 *M* sodium chloride and appeared in fraction 1.

DISCUSSION

This procedure provides a simple rapid method for the DEAE-cellulose chromatography of small samples of acidic glycoproteins which can be readily adapted for the routine, simultaneous, processing of multiple samples. In previous chemical and histochemical studies [8,9] we have shown that in many tumours of the colon there is a marked reduction in the degree of O-acetylation of the epithelial glycoprotein sialic acids; use of this method will facilitate the further analysis of such glycoproteins. Although the procedure has been applied primarily to extracts of fixed tissues it should be applicable to the separation of any mixture of acidic glycoproteins which can be fractionated by conventional DEAE-cellulose chromatography and therefore should be of value in many biochemical and biomedical investigations.

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