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PREPARATIVE FRACTIONATION OF CARBOHYDRATE-RICH COMPONENTS PRESENT IN GERM-FREE RAT INTESTINAL MUCIN BY GEL FILTRATION

COMPARISON OF DYNOSPHERES® XP-3505 AND SEPHAROSE CL 4B

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SUMMARY

Fractionation of the carbohydrate-rich mucin present in the intestines of germ-free rats has been achieved on Dynospheres XP-3505. Comparison with Sepharose CL 4B shows that the separation on Dynospheres XP-3505 is better and quicker. The carbohydrate composition of the fractions show little difference from that of the crude material.

INTRODUCTION

The epithelial surface of the mammalian intestinal tract is protected by a flowing layer of mucus continuously produced by secretory cells of the intestinal mucosa. This layer is part of the barrier through which substances have to pass when absorbed from the intestinal tract. It also serves as a mechanical protective barrier and contributes to maintain a relatively constant pH and ion concentration in the environment of the tender microvilli[1]. The intestinal mucin secretions are degraded by the microflora of the digestive tract[2,3]. This fact complicates the isolation and the study of the native product. By the use of animals devoid of any microflora in the digestive tract, intestinal mucin can be isolated in a yield better than that obtained from conventional animals. Prior to structural studies of the carbohydrate moiety of this mucin, the product is digested with pronase.

The acidic part of the retentate after dialysis was precipitated as a cetyltrimethylammonium (CTAB) complex and further purified on a DEAE Sephadex A25 anion-exchange column. The fractions containing the major part of neutral sugars and sialic acid were subjected to partial separation on Sepharose 4B[1]. For more detailed studies on the carbohydrate moiety of the rat intestinal mucin further separation of the material is necessary, and the high-molecular-weight (HMW) fraction was rechromatographed on Dynospheres XP-3505 and Sepharose CL 4B in order to find a suitable separation medium for the actual material.

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EXPERIMENTAL

The starting material was prepared as described earlier[1]. Gel filtration was performed on two different columns:

(i) Sepharose CL 4B, (Pharmacia, Uppsala, Sweden) 37 × 1 cm I.D. The column was eluted with 0.1 *M* ammonium hydrogen carbonate at a flow-rate of 0.3 ml/min, controlled by a Pharmacia P-1 pump. Fractions of 1.2 ml were collected using a LKB Redirac 2112 fraction collector. The carbohydrate profile was determined by the method of Dubois *et al.*[4] and the protein profile by the Lowry[5] method. The results are presented in Fig. 1.

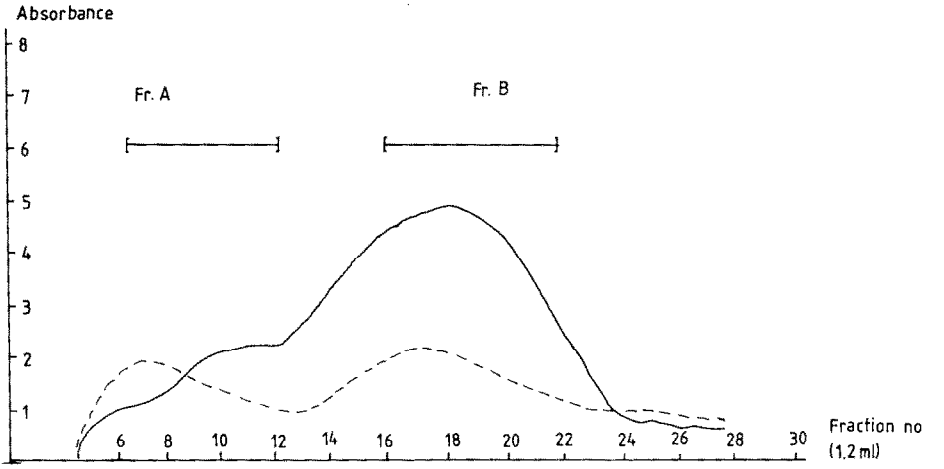

Fig. 1. Gel filtration on Sepharose CL 4B of HMW fraction from the rat intestinal mucus prepared as described in the text. Fractions A and B were rechromatographed on Dynospheres XP-3505. The continuous line is the carbohydrate[4] profile, and the dashed line is the protein profile[5]. Flow-rate, 0.3 ml/min; eluent, 0.1 *M* ammonium hydrogen carbonate.

(ii) Dynospheres XP-3505, (Dyno, Lillestrøm, Norway) 44 × 1 cm I.D. The column was eluted with 10 m*M* ammonium hydrogen carbonate at a flow-rate of 2 ml/min. The column was coupled to a LKB 2150 HPLC pump, and the eluent was monitored by an Optilab 5902 refractometer. The column was also coupled to a Waters HPLC system (510 pump, 481 UV detector, 840 data system) and OD 280 was monitored throughout the elution of the column (Fig. 2).

Characteristics of Dynospheres XP-3505

The pressure–flow curve is linear over a wide range, *i.e.* a flow of 2000 ml/cm^2 × *h* gives a pressure of 30 bar. The medium is stable in water, 1 *M* hydrochloric acid, 0.1 *M* sodium hydroxide, 24% ethanol and 8 *M* urea.

Dextrans T 2000, T 250, T 70 and T 40 were chromatographed on the Dynospheres XP-3505 column. Dextran T 2000 was eluted with the void volume (6.7 ml); the other dextrans were eluted as indicated by arrows on Fig. 2.

Fractions A and B (Fig. 1) and fractions 1 and 2 (Fig. 2) were, after dialysis

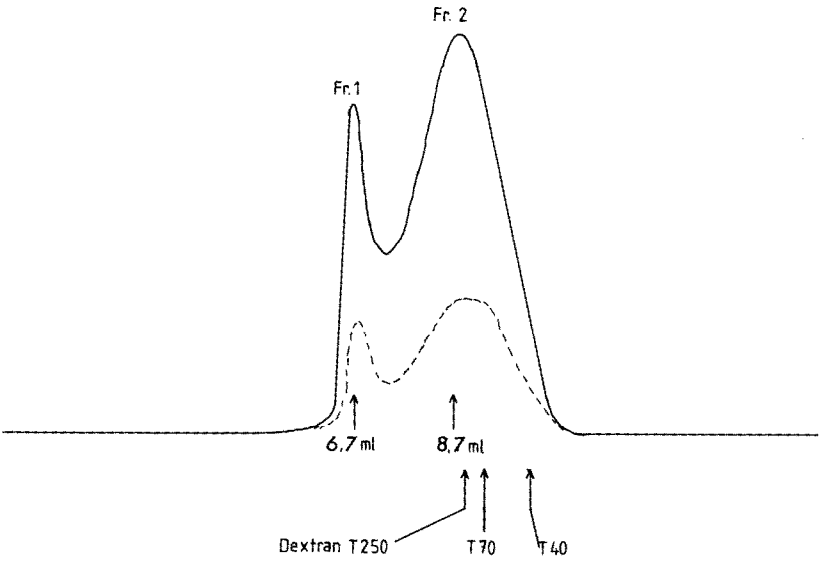

Fig. 2. Gel filtration on Dynospheres XP-3505 of HMW fraction from the rat intestinal mucus prepared as described in the text. Fraction 1 and 2 were rechromatographed on Dynospheres XP-3505. The continuous line is from refractive index detection, and the dashed line is the optical density at 280 nm. Flow-rate, 2 ml/min; eluent, 10 m*M* ammonium hydrogen carbonate.

(cut of 12.000) and freeze drying, rechromatographed on the Dynospheres XP-3505 column with conditions as described above. The results are presented in Figs. 3 and 4. The carbohydrate composition of the starting material and fractions A, B, 1 and 2 were determined by gas chromatography (GC) of the TMS derivatives of the methylglycosides[6] of the sugars present in the samples. GC was performed on DB-5 fused-silica column, (25 m × 0.25 mm I.D.), with the following programme: injection temperature 140°C followed by an increase of 1°C/min to 170°C, then an increase of 6°C/min to 250°C. The method does to separate glycolyl and acetylneuraminic acid (sialic acids). The carbohydrate composition is presented in Table I.

RESULTS AND DISCUSSION

The object of this work was to find a suitable medium for preparation of pure mucin present in the rat intestinal mucus. Chromatography of the HMW fraction on the Sepharose CL 4B column and on the Dynospheres XP-3505 column was performed, and the results are presented in Fig. 1 and 2, respectively. The separation of compounds present in the HMW fraction of the rat intestinal mucus appears from these results to be achieved better on the Dynospheres XP-3505 column than on the Sepharose CL 4B column.

A comparison of fraction A (Fig. 1) and fraction 1 (Fig. 2), when rechromatographed on Dynospheres XP-3505, demonstrates that A contains a substantial part of B, whereas 1 is almost devoid of 2. Fraction B and fraction 2 appear to constitute the same portion of HMW.

TABLE I

CARBOHYDRATE COMPOSITION OF THE VARIOUS FRACTIONS OBTAINED FROM THE RAT INTESTINAL MUCUS

Expressed as percentages of the total carbohydrate present.

	Fucose	*Mannose*	*Galactose*	*Glucose*	*N-Acetylglucosamine*	*N-Acetylgalactosamine*	*Sialic acid*
Starting material	6.7	1.1	23.6	1.8	17.5	25.6	23.6
Fraction 1, Dynospheres	8.2	0.8	22.8	–	20.7	28.4	19.1
Fraction A Sepharose	8.1	0.4	23.2	–	17.7	28.2	21.3
Fraction 2, Dynospheres	9.5	1.3	22.2	–	19.9	25.9	21.2
Fraction B Sepharose	12.1	1.2	22.3	–	15.1	25.1	24.3

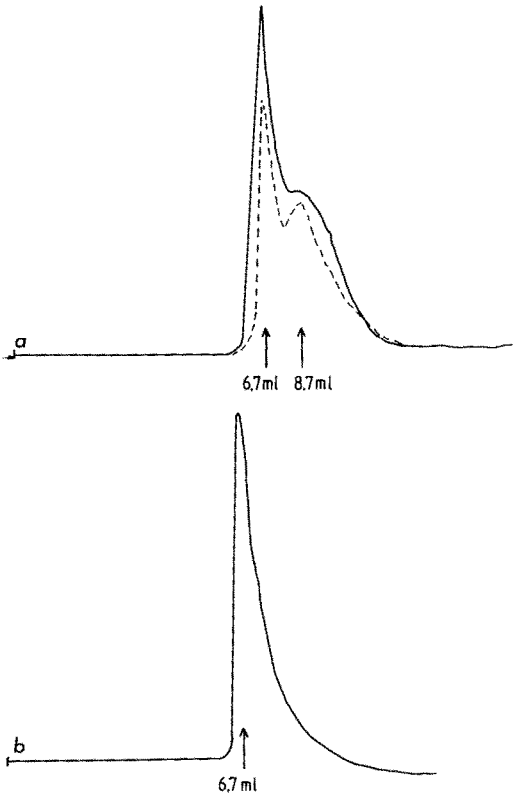

Fig. 3. (a) Gel filtration of fraction A (Fig. 1) on Dynospheres XP-3505. (b) Gel filtration of fraction 1 (Fig. 2) on Dynospheres XP-3505. Conditions as given for Fig. 2.

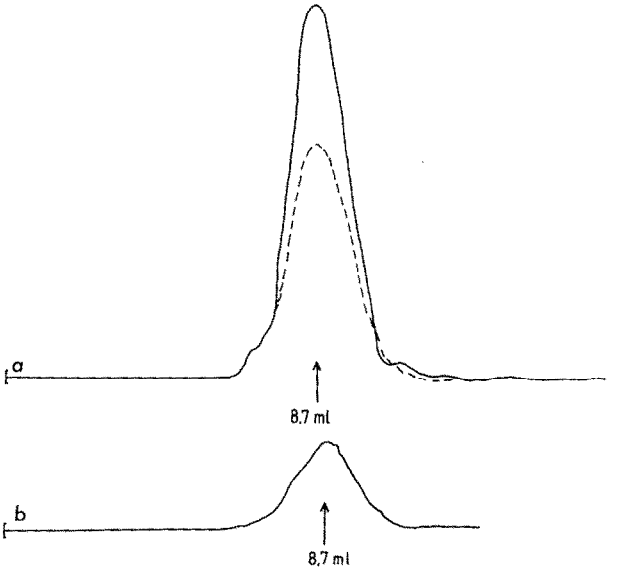

Fig. 4. (a) Gel filtration of fraction B (Fig. 1) on Dynospheres XP-3505. (b) Gel filtration of fraction 2 (Fig. 2) on Dynospheres XP-3505. Conditions as given for Fig. 2.

Fraction 1, being identical with the first peak of fraction A, is eluted with the void volume, whereas fraction 2, identical with fraction B, appears to have a molecular weight of 280 000 with reference to dextrans.

The carbohydrate compositions of the HMW fraction and subfractions obtained by separation on Sepharose CL 4B and Dynospheres XP-3505 show little difference (Table I), which is in accord with results obtained from previous separation studies on the rat intestinal mucus[1].

CONCLUSION

Our results show that Dynospheres XP-3505 is a more suitable gel filtration medium for separation of components present in rat intestinal mucion than Sepharose CL 4B. The Dynospheres XP-3505 column can be eluted at a higher flow-rate (at least 6 times) than the Sepharose CL 4B column, and gives better separation.

Large-scale gel filtration on Dynospheres XP-3505 will be time-saving compared with gel filtration media based on other matrices.

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