Journal of Chromatography, 463 (1989) 445–451
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROM. 21 068

Note

Analysis of mucin by isotachophoresis

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(First received July 22nd, 1988; revised manuscript received October 24th, 1988)

The glycoprotein mucin is defined according to ref. 1 as the fraction precipitated by approximately 60% ethyl alcohol from the supernatant liquid after pepsin–hydrochloric acid digestion of hog stomach linings. A number of tests are presented[1] in order to characterize mucin and to guarantee a constant quality when producing porcine mucin.

During a search for a method which could provide a fingerprint of mucin, *cf.*, ref. 2, and a quantitative assay, we selected isotachophoresis as a suitable alternative to the classical methods[1]. This technique has been applied to the analysis of proteins and peptides[3,4]. In our laboratory, isotachophoresis has been used previously for the analysis of methotrexate[5].

MATERIALS AND METHODS

Apparatus

Isotachophoretic experiments were performed in the apparatus provided with UV absorption and conductivity detection as described by Everaerts *et al.*[6]. The separation capillary was approximately 200 mm × 0.2 mm I.D. The electric current was stabilized at 0.06 mA. The electrolyte system used in the isotachophoresis of mucin and other proteins is specified in Table I. The isotachophoretic apparatus was equipped with a Linseis three-channel recorder Type LS34, registering the UV signal (50 mV), the conductivity signal (2.5 mV) and the differentiated conductivity signal. The paper velocity was 20 mm/min. The UV signal was also recorded on a Hewlett-Packard integrator ATT3 (attenuation 3). The paper velocity was 25 mm/min. In order to monitor the whole isotachophoretic separation, use was made of a barrier filter (Longpass 515) and a mercury lamp (HBO 50) with a Shortpass 490 filter. Filters were from Schott (Mainz, F.R.G.).

Reagents

Reagents used were of analytical grade. Water was purified by Millipore ultrafiltration. Mowiol 8-88 was kindly donated by Hoechst-Holland (Amsterdam, The Netherlands) and purified by ion-exchange chromatography (Merck V; Merck, Darmstadt, F.R.G.). Fluorescein isothiocyanate was from Merck.

0021-9673/89/$03.50

TABLE I

OPERATIONAL SYSTEM FOR MUCIN ANALYSIS

Parameter	*Electrolyte*	
	Leading	*Terminator*
Anion	Cl^-	Glycine
Concentration (*M*)	0.01	0.01
Counter ion	Tris	Tris
pH	7.2	8.5
Additive	0.05% Mowiol	
Solvent	Water	Water

Protein preparations

Mucin reference material was freeze-dried batch 315 from A/S Orthana Kemisk Fabrik (Kastrup, Denmark). This batch was characterized by, *inter alia*, UV, IR and ^{13}C Fourier transform (FT)-NMR spectroscopy. Mucin from A/S Orthana is porcine gastric mucin. Further, 3.5% mucin solution from A/S Orthana was batch 230388; pepsin (batch 23326) and peptone 0-24 (batch 4913) were also from A/S Orthana. Bovine albumin was from Sigma (St. Louis, MO, U.S.A.), A-8022, Lot 51F-0320.

Procedure

In order to obtain a qualitative analysis of mucin (or another protein), 1 μl of a 3.5% solution was injected between the leading and terminating electrolytes. The isotachopherogram was compared with that of a 3.5% solution of reference batch 315. For the quantitative analysis, the zone length of the sample solution was compared with that of two reference solutions of batch 315. The beginning and end of a zone can be checked by using the differentiated signal. The percentages indicated below denote g per 100 ml. The isotachophoretic analysis of a mucin sample may take up to 1 h.

RESULTS AND DISCUSSION

Mucin solutions may have a high viscosity while, in general, proteins may adhere to the capillary tube during isotachophoresis[6]. In order to check whether our analysis was in accordance with the isotachophoretic requirement: mobility leading electrolyte > mobility sample > mobility terminating electrolyte, the complete isotachophoretic process was monitored. The mucin was labelled by fluorescein isothiocyanate and the process was inspected visually upon UV irradiation. All proteins participated in the isotachophoresis. Also from the conductometric end signal which was equal to that of the terminator, it became clear that no protein components were migrating in the terminator.

In Fig. 1, isotachopherograms of mucin, pepsin, peptone and albumin are presented as recorded by the Linseis recorder. The protein zones are marked in the figures. Inspection of this figure shows that the isotachopherogram of mucin is quite different from that of pepsin, peptone or albumin. Mucin and pepsin look rather complex protein mixtures. The isotachopherograms of peptone and albumin indicate a limited number of compounds. Compared with the isotachopherograms of possible

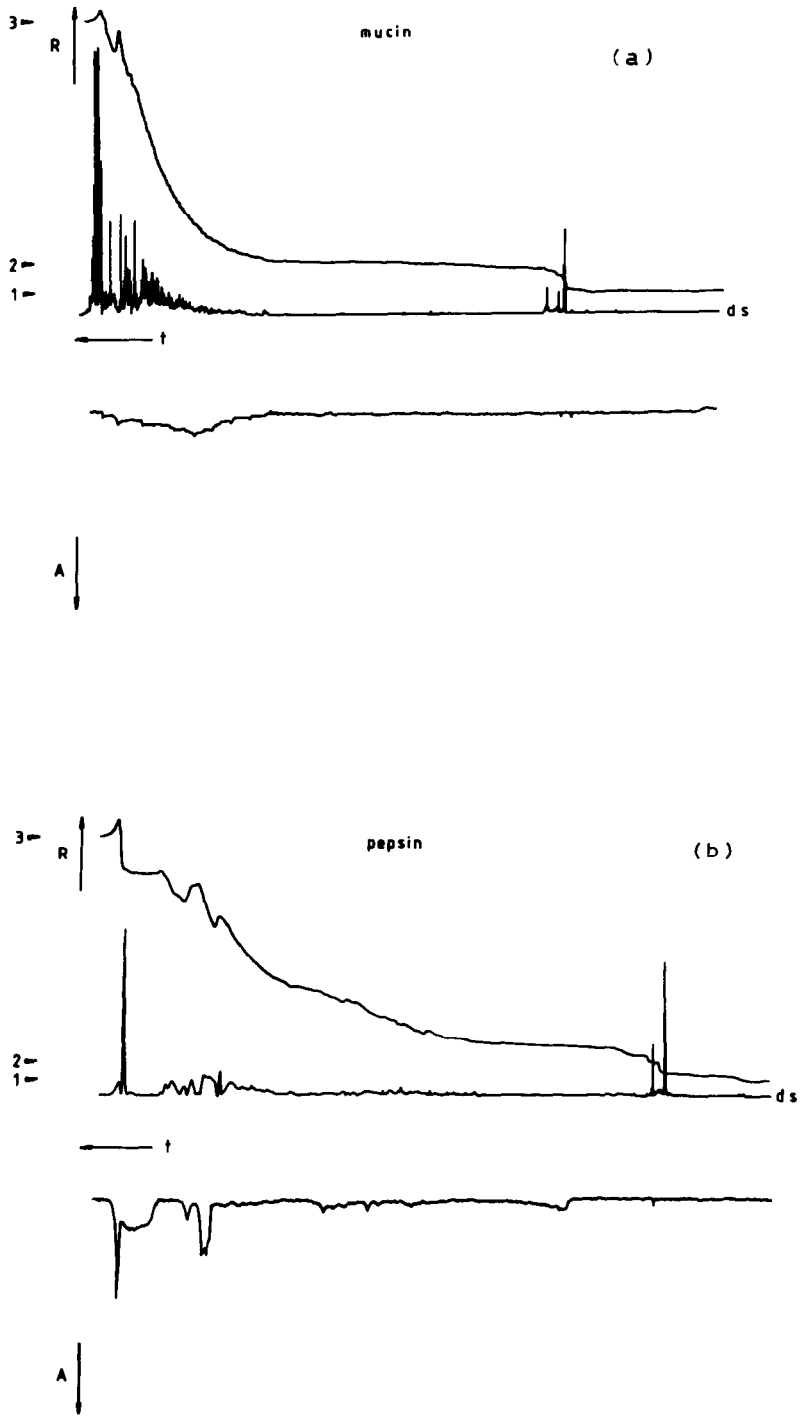

Fig. 1.

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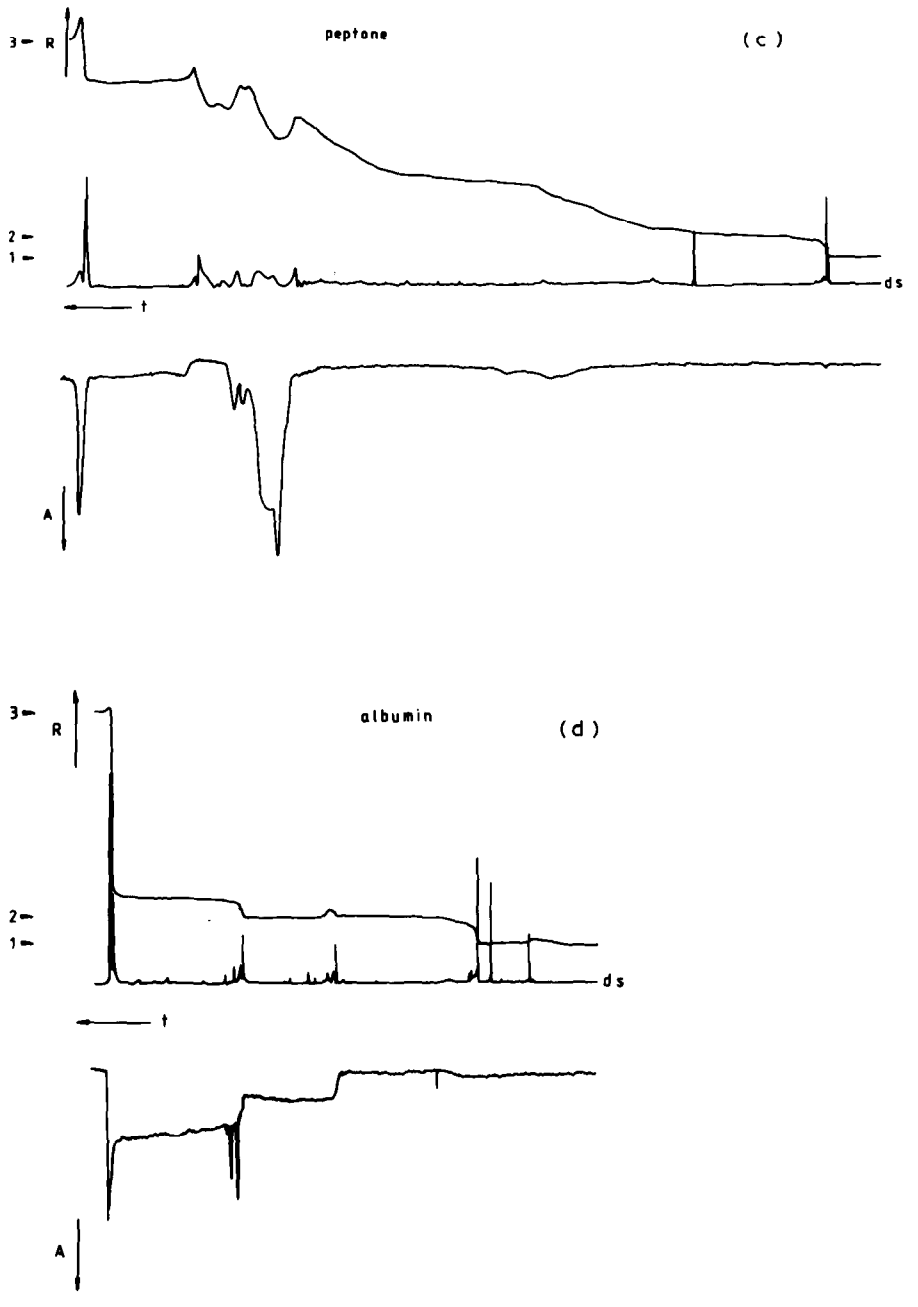

Fig. 1. Isotachopherograms of 3.5% (w/v) solutions of mucin, pepsin, peptone and albumin as recorded by the Linseis recorder. The nature of the anions in the zones is indicated by their resistance level, *R*, or UV absorption, *A*. The zone length indicates the quantity of anion passing the detector. Also shown is the differential signal (ds) of the conductometer, facilitating the determination of the zone length. Time is from right to left. Step heights: 1 = leading electrolyte; 2 = non-UV-absorbing ions from protein sample; 3 = terminating electrolyte; the protein zone is between 2 and 3.

TABLE II

COMPOSITION OF A 3.5% MUCIN SOLUTION

Data from A/S Orthana.

Component	*Content (g/100 ml)*
Mucin	3.5
Na^+	0.080
K^+	0.070
Ca^{2+}	0.025
Mg^{2+}	0.015
HPO_4^{2-}	0.022
Cl^-	0.120

impurities resulting from the manufacturing process (pepsin and peptone) or to a completely unrelated protein (albumin), the isotachopherogram of mucin appears to possess a high specificity.

From Fig. 1 it is clear that non-UV absorbing species are present between the leading zone and the protein zone. This was partly known from the composition of mucin which is presented for a 3.5% solution in Table II. Isotachophoresis may lead to further definition of mucin and related peptides. For a discussion on HCO_3^- which is also present in the system used, see ref. 6. This ion is not shown in the isotachophoretic traces in the figures.

Isotachophoresis was subsequently used to establish the mucin identity of mucin production batches. The isotachopherograms obtained from samples taken during the manufacturing process were identical to that of mucin in Fig. 1.

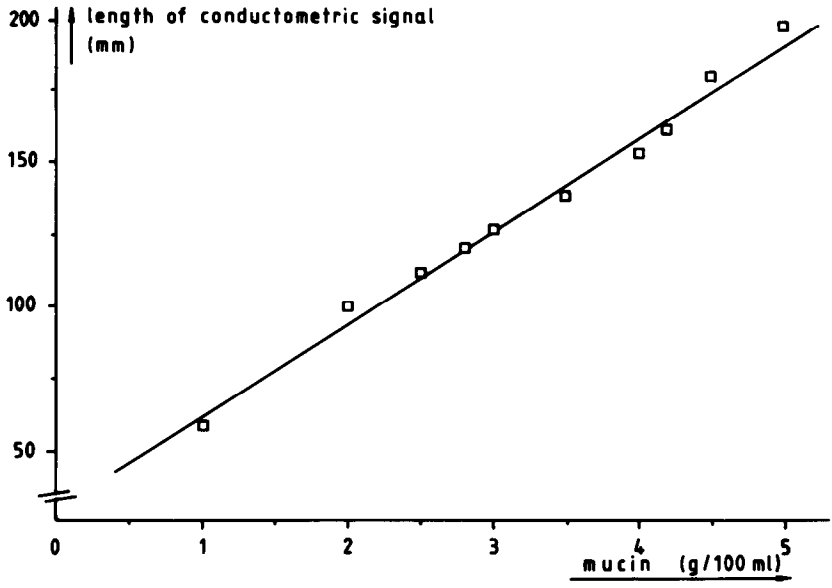

Fig. 2. Zone length of the conductometric signal *versus* concentration for 1–5% mucin solutions.

The zone length found in an isotachopherogram is a measure of the amount of anions in the zone. In the present study, a linear relationship was found between the concentration of mucin and the zone length in the range 1–5% mucin (concentrations 1.0, 2.0, 2.5, 2.8, 3.0, 3.5, 4.0, 4.2, 4.5 and 5.0%; correlation coefficient 0.994). The daily variation was 1.5% ($n = 12$). An example of a calibration graph is presented in Fig. 2. Fig. 3 gives an impression of the UV part of the isotachopherograms from a calibration graph. In this figure only the UV-absorbing protein part of the isotachopherogram is presented as recorded on the HP ATT3.

Using batch 315 (freeze-dried material) as a reference, the mucin content of a production batch of mucin solution (388) was determined. Two reference solutions of 3.5% mucin were prepared. These were measured as well as the sample solution in duplicate on 2 days: batch 388 showed a mucin content of 3.1 ± 0.1%.

Isotachophoresis has proven to be a suitable method for the qualitative as well as

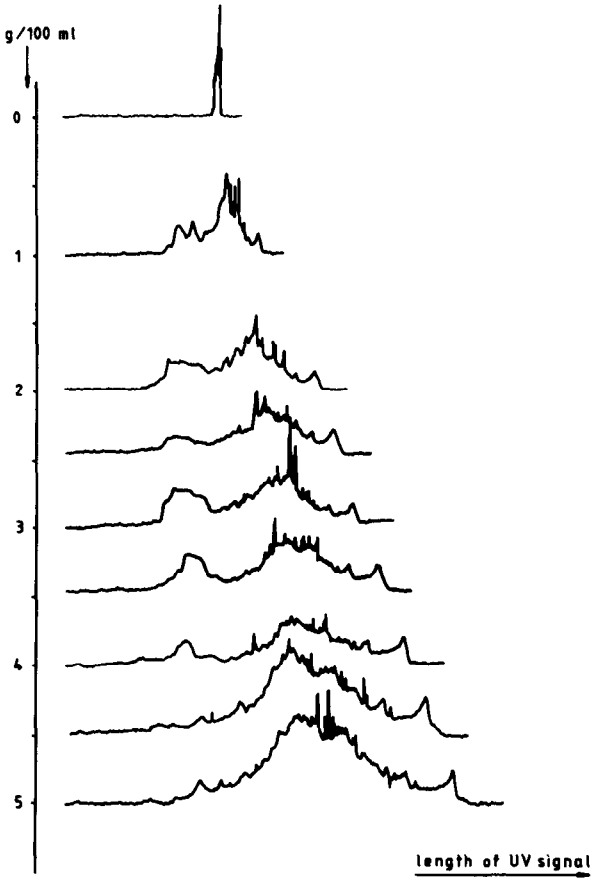

Fig. 3. UV signal of mucin samples from a calibration graph, showing a fingerprint comparison of increasing mucin concentrations. This figure gives the UV-absorbing protein part of the isotachopherogram recorded by the HP ATT3. Zone length was measured by using the differential signal of the conductometer. Time is from left to right.

the quantitative analysis of mucin in solution. It provides a new and independent analytical method to assay mucin and may as such be a modern supplement to the traditional methods[1].

ACKNOWLEDGEMENTS

We thank Mr. E. Balslev of A/S Orthana, Kastrup, Denmark for supporting this study, Ruud van der Geest and Riemer Runia for their technical assistance, Jan Slats for his help during the UV monitoring of the isotachophoretic process and Dr. O. M. J. Driessen for editing the manuscript.

REFERENCES

1 *Tests and Standards for New and Non-official Remedies*, The Chemical Laboratory of the American Medical Association, JB Lippincott Company, Philadelphia, PA, 1953.
2 E. Moczar and D. Waldron-Edward, *J. Chromatogr.*, 181 (1980) 108–114.
3 P. Stehle, H.-P. Bahsitta and P. Fürst, *J. Chromatogr.*, 370 (1986) 131–138.
4 D. Del Principe, *J. Chromatogr.*, 342 (1985) 285–292.
5 O. Driessen, H. Beukers, L. Belfroid and A. Emonds, *J. Chromatogr.*, 181 (1980) 441–448.
6 F. M. Everaerts, J. L. Beckers and T. P. E. M. Verheggen, *Isotachophoresis, Theory, Instrumentation and Application*, Elsevier, Amsterdam, 1976.