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MICROPREPARATIVE ISOTACHOPHORETIC ELECTRODESORPTION OF MONOCLONAL ANTIBODIES FROM AN AFFINITY ADSORBENT

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SUMMARY

The principle of isotachophoretic desorption of proteins from an affinity sorbent was used for the preparative isolation of monoclonal antibodies against transferrin, under a cationic regimen. Electrodesorption was carried out on an apparatus of our own construction with a photometric detector and a detector of potential gradient. The apparatus was provided with a sorption element filled with an exchangeable affinity active sorbent. The construction of the apparatus and the procedure suitable for the isolation of the antibody of the IgG type are described. The possibility of repeating the sorption–desorption cycle at least sixteen times was demonstrated, with relatively good yields of the protein.

INTRODUCTION

The introduction of the principle of affinity or bioaffinity sorption resulted in a substantial increase in the selectivity of separation, and the combination with electrodesorption[1,2] also increased the possibility of isolating labile proteins in a nondestructive way.

The commonest method of electrodesorption in a homogeneous electrolyte[3,4] is generally time consuming and/or affords dilute protein solutions. If isoelectric focusing is used, *i.e.*, electrodesorption in a pH gradient[5,6], more concentrated solutions are obtained, but the risk of damaging the activity of the protein under the influence of extreme pH values is high. This applies mainly to the electrodesorption of proteins with more extreme isoelectric points. A more detailed survey and an analysis of the electrodesorption methods used so far was given in an earlier paper[7].

Using modelling of the electrodesorption procedure by isotachophoresis in an analytical form, the method of nondestructive desorption with subsequent concentration in one process may be optimized. From the point of view of time economy and with respect to protein stability, it is not necessary to allow the process of isotachophoretic desorption to proceed to the steady state, but the process of isotachophoretic desorption may be interrupted in the course of the transient state. The instruments for electrodesorption[8–12] are usually provided with a semipermeable membrane, a polyacrylamide gel layer, etc., so that the required concentration of the

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desorbed macromolecular component can take place. The newly developed apparatus allows the isolation of milligram amounts of isotachophoretically desorbed protein at a resulting concentration of about 0.5% (w/v) with an average time of 2.5 h per sorption–desorption cycle.

EXPERIMENTAL

Chemicals

Analytical-reagent grade chemicals were used. Hydrochloric acid (Normanal), potassium hydroxide and sodium azide were obtained from Lachema (Brno, Czechoslovakia), 6-aminocaproic acid (6-ACA) from Koch-Light (Colnbrook, U.K.), 2-(N-morpholino)ethanesulphonic acid (MES) from Calbiochem (San Diego, CA, U.S.A.) and polyethylene glycol (MW 20,000) from Serva (Heidelberg, F.R.G.). Mouse monoclonal antibody to porcine transferrin (Ab), IgG1 in PBS with 0.1% sodium azide and polyethylene glycol terephthalate microgranular carrier (Sorsilen) coated with porcine transferrin (Insoltransferrin) were kindly supplied by Dr. F. Franěk (Institute of Molecular Genetics, Czechoslovak Academy of Sciences, Prague, Czechoslovakia).

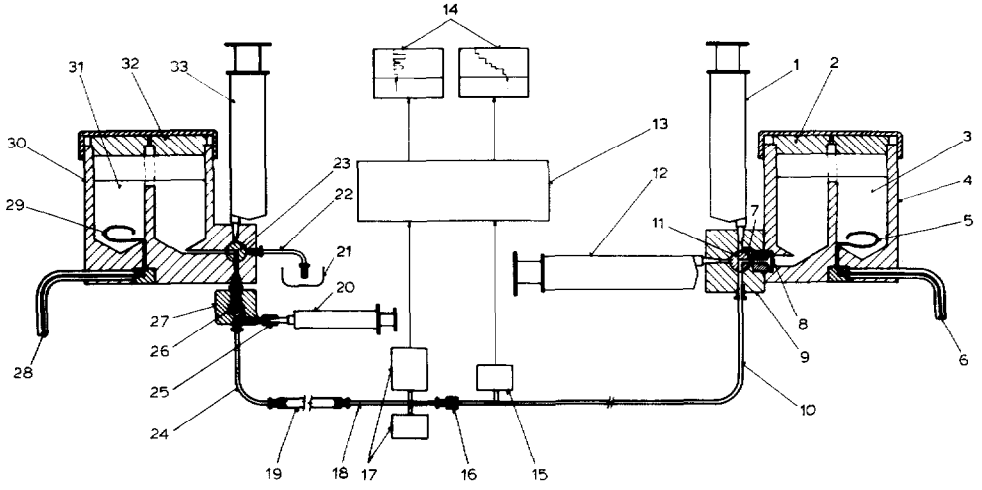

Fig. 1. Apparatus for preparative isotachophoretic desorption. 1 = Polypropylene 20-ml syringe for the leading electrolyte; 2 = cover of the electrode compartment for the leading electrolyte; 3 = leading electrolyte; 4 = electrode vessel for the leading electrolyte; 5 = Pt electrode; 6 = high-voltage cable; 7 = deaeration channel; 8 = semipermeable membrane (Kuprophan); 9 = body of the valve of the leading electrolyte; 10 = PTFE tubing, I.D. 1 mm; 11 = PTFE core of the valve of the leading electrolyte; 12 = polypropylene 20-ml syringe for filling the capillary and rinsing the sample; 13 = electronic set of the UV detector and the potential gradient (PG) detector; 14 = recorders of the UV and PG signals; 15 = PG detector; 16 = PTFE coupling; 17 = source and sensor of the UV detector; 18 = PTFE tubing, I.D. 1 mm; 19 = polyethylene tubing, I.D. 3 mm; 20 = polypropylene syringe for the sample and deaeration; 21 = waste reservoir; 22 = PTFE tubing, I.D. 1 mm; 23 = PTFE core of the valve for the terminating electrolyte; 24 = PTFE tubing, I.D. 1 mm; 25 = needle valve joint; 26 = segment with the affinity sorbent; 27 = body of the sorption element; 28 = high-voltage cable; 29 = Pt electrode; 30 = electrode vessel for the terminating electrolyte; 31 = terminating electrolyte; 32 = cover of the vessel for terminating electrolyte; 33 = polypropylene 20-ml syringe for the terminating electrolyte.

Electrolyte system

In the leading electrolyte, the leading ion was 0.005 mol l^{-1} K^+, the counter ion MES and the pH 5.7. In the terminating electrolyte, the terminating ion was 0.01 mol l^{-1} 6-ACA, counter ion Cl^- and the pH 5.2.

Apparatus

Analytical evaluation of desorbed protein was carried out on an analytical capillary isotachophoresis instrument of our own construction, according to Kašička and co-workers[7,13,14].

The apparatus is composed of electrode compartments with valves, a system of capillaries with disconnectable joints, a UV-photometric and potential gradient (PG) detection system provided with a recording device, a peristaltic micropump and a power supply of high voltage (up to 20 kV) with a current stabilizer up to 500 μA and recording of the output voltage. A general scheme is shown in Fig. 1. Between the electrode compartment of the terminating electrolyte and the capillary system is a sorption element with an effective I.D. of 9 mm in which a disc with the affinity adsorbent is located. A longitudinal cross-section of the sorption element is shown in Fig. 2. In order to give a better idea, an expanded view of the sorption element is shown in Fig. 3.

The detection system consists of a UV absorption detector with a low-pressure mercury lamp (Tesla, Prague, Czechoslovakia) with a UG 5 filter for the 253.7 nm wavelength and a potential gradient detector of our own construction[15] with an all-PTFE system of I.D. 1 mm, with Pt–Ir implanted detection electrodes at a distance of 0.15 mm in the direction of the electrical field.

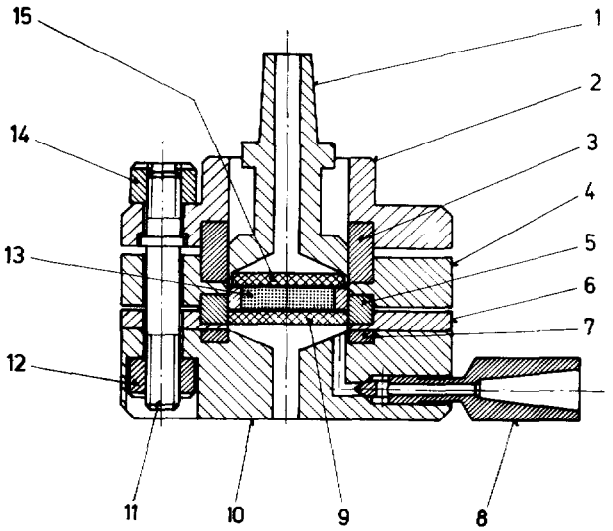

Fig. 2. Longitudinal cross-section of the sorption element. 1 = Connector closure of the sorption element; 2 = upper segment; 3 = silicone rubber seal; 4 = central segment; 5 = silicone rubber seal; 6 = supporting segment; 7 = silicone rubber seal; 8 = needle valve joint for the syringe; 9 = polypropylene porous disc; 10 = lower segment; 11 = clamp; 12 = lower nut of the clamp (stainless steel); 13 = affinity sorbent; 14 = upper nut of the clamp (connector); 15 = polypropylene porous disc.

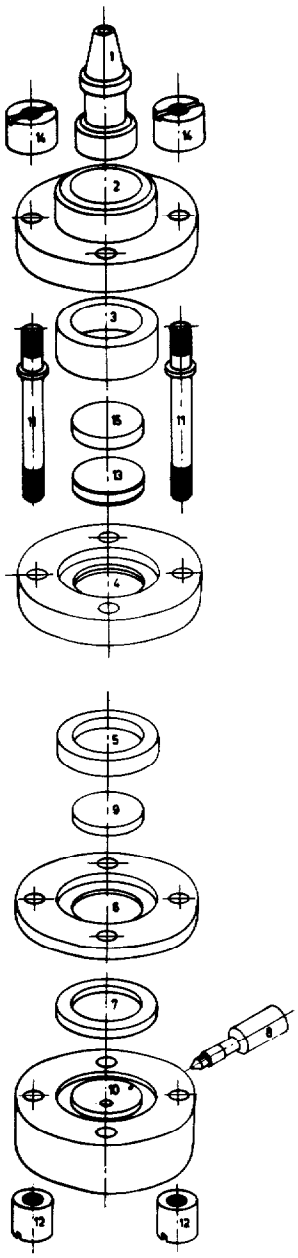

Fig. 3. Expanded view of sorption element. The individual parts correspond to the numbers in Fig. 2.

Procedure

Equilibration of the affinity sorbent. A 0.13-ml volume of the sorbent is washed in the sorption element with 6 ml of 0.1% (w/v) of polyethylene glycol (MW 20,000) in the leading electrolyte, using the peristaltic pump, at a flow-rate of 18 ml h^{-1}, and the affinity sorbent is washed with 3 ml of the leading electrolyte at the same flow-rate.

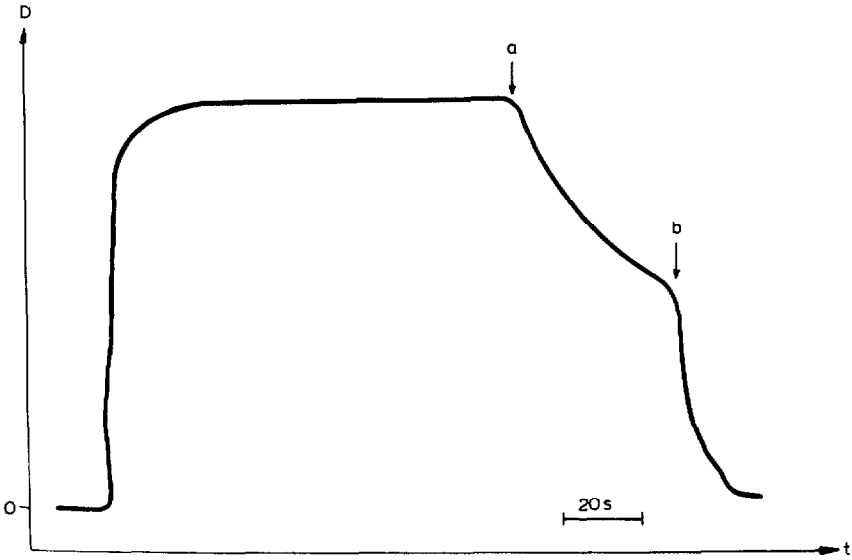

Fig. 4. Record of the course of electrodesorption of the monoclonal antibody by the 254 nm UV detector. T = transmittance in 1 mm I.D. PTFE tubing; D = absorptance, where $D = (1 - T)$; t = time. A 0.25 ml of 1.05% (w/v) sample of monoclonal antibody in phosphate buffered saline (PBS) containing 0.1% of sodium azide was diluted to 6 ml with the leading electrolyte, followed by adsorption at a through-flow of 18 ml h^{-1} for 20 min, then washed with 3 ml of the leading electrolyte at the same through-flow rate. The volume of the affinity sorbent was 0.13 ml, time of electrodesorption 108 min, current 130 μA and desorbent amount of the immunochemically active antibody 0.92 mg.

Sorption of the antibody. A solution of the antibody Ab is pumped from below into the sorption element 27 (the numbering of the parts of the apparatus refers to Fig. 1). After its passage through the sorbent the solution, depleted of the affinity active antibody, is collected in the waste reservoir 21. For more details, see Fig. 4.

Washing of the sorbent. Using the micropump connected with the coupling 25 the sorbent is washed with 3 ml of the leading electrolyte and freed from the affinity inactive components.

Isotachophoresis. The valves of the leading (11) and the terminating (23) electrode compartments are set to a position in which the passage of the current through the separation space is made possible, the needle-valve coupling 25 is closed, the high-voltage power supply is switched on and the required value of the stabilized current is set. The course of the clamp voltage of the HV power supply and the course of the voltage on the PG detector 15 are recorded, and also the course of the transmittance or absorbance of the UV detector 17.

As soon as the zone of the desorbed antibody has reached the PG detector, which is manifested by a distinct increase in the signal, the electrodesorption is terminated by switching off the current. The shape and the position of the desorbate zone can be read from the record of the UV detector 17 (see also the record in Fig. 4). The zone is located between the UV detector and the PG detector.

The valve of the leading electrolyte 11 is switched over to the filling position and the separation capillary 10 communicates with the syringe 12 filled with air. The joint between the PTFE tubing 18 and the polyethylene tubing 19 is disconnected

and the solution of the desorbed antibody is pushed out with air by means of the syringe 12 into a micro test-tube.

RESULTS AND DISCUSSION

For the isolation of the labile proteins the above-described apparatus was used, with the sorption element which allows a change in the volume of the affinity sorbent within certain limits. After experience with the analytical method of isotachophoretic desorption[7] a 2 mm layer thickness of the sorbent was selected, corresponding to a volume of 0.13 ml. This layer thickness is a compromise between the total capacity of the sorbent and the efficiency of the desorption. When the sorbent layer is thicker, electrodesorption is slower, the yields decrease, the time of electrodesorption is prolonged and it is necessary to increase the volume of the separation space, i.e., to elongate the section of the polyethylene tube of I.D. 3 mm.

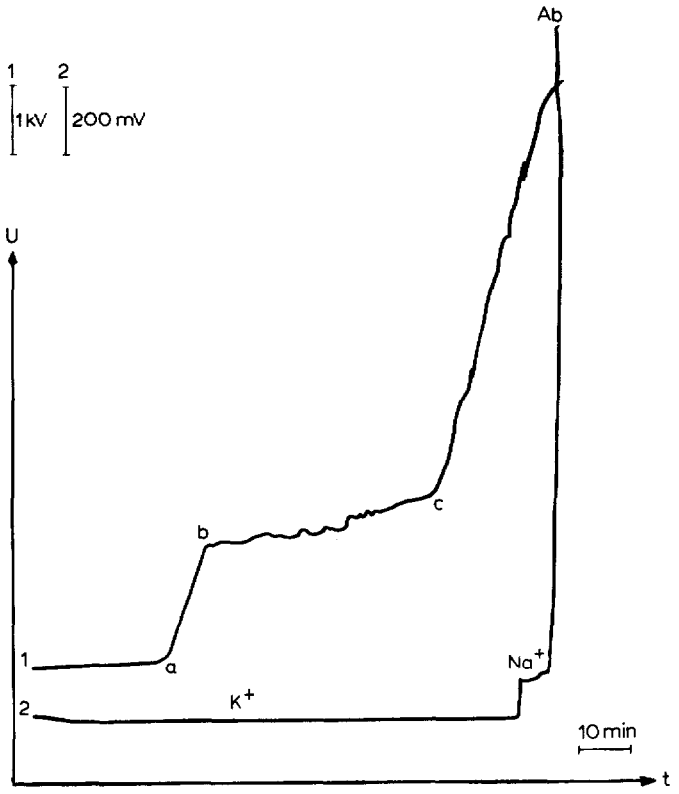

Fig. 5. Passage of zone boundaries of desorbed protein through the separation spaces. 1, Clamp voltage–time profile of the current-stabilized high-voltage supply. Changes in the slope of the recorded curve indicate the passage of the desorbed antibody zone boundary through different sizes of tubing: a–b, from the sorption element of 9 mm I.D. to 1 mm I.D. tubing; b–c, from 1 mm I.D. tubing to 3 mm I.D. tubing; c, from 3 mm I.D. tubing to the 1 mm I.D. tubing with UV and PG detectors. 2, The record of the unshielded PG detector indicates the passage of the zones of K^+, Na^+ and Ab (monoclonal antibody). After the passage of the boundary of zone Ab through the PG detector, electrodesorption is ended. For details, see the text.

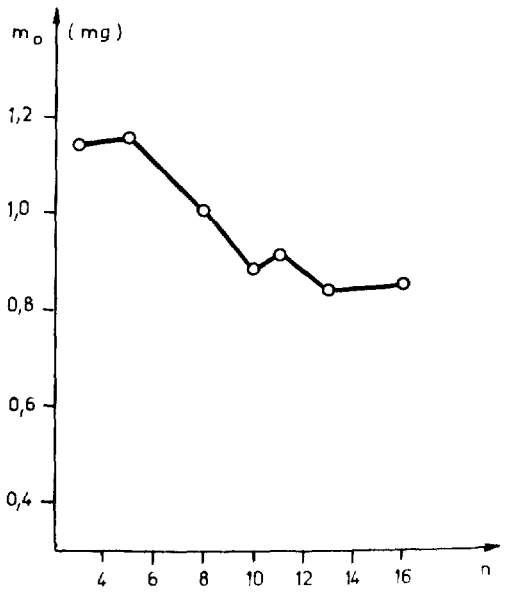

Fig. 6. Yield of preparative isotachophoretic desorption of monoclonal antibody as a function of the number of sorption–desorption cycles. m_0 = Amount of desorbed antibody (mg); n = number of sorption–desorption cycles. The cycles in which sodium azide + NaCl was used as a conserving agent for the affinity sorbent have been omitted.

From the point of view of preparative use of isotachophoretic desorption, the question of the repeatability of the experiment without exchange of the affinity sorbent is important. In order to check this point, the process was repeated in a series of 16 cycles in which a new solution of the impure antibody containing sodium azide was always sorbed. When interrupting the series by electrodesorption overnight the affinity sorbent in the sorption element was always washed and filled with the conservation solution, *i.e.*, 0.15 *M* NaCl containing 0.1% (w/v) of sodium azide (final concentration). The yields of the active antibody obtained in a series of electrodesorptions are shown in Fig. 6. After an initial decrease, the maximum capacity of the sorbent (*i.e.*, 1.2 mg of the protein) was achieved in the second to fifth cycles. From the results it also follows that even after 16 cycles 74% of the maximum capacity of the affinity sorbent is still preserved.

The non-destructive procedure of preparative isotachophoretic desorption will evidently be utilisable not only for the affinity couple immobilized antigen–antibody, but also for other affinity couples with a soluble ionogenic component. Further applications of preparative isotachophoretic desorption in the milligram range and further improvements to the apparatus will be the subject of continuing study.

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