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Note

Desorption isotachophoresis —quantitative characterization of sorption and desorption conditions

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Electrophoretic desorption (electrodesorption)[1] has proved to be a valuable method for releasing the bioaffinity adsorbed soluble complementary component (ligate)[2] from the affinity sorbent, especially in the case of very strong complexes (*e.g.*, antigen–antibody) where the usual chemical releasing methods (changes of pH, ionic strength, addition of chaotropic reagents and detergents) cause partial or even complete loss of biological activity of the desorbed ligate.

So far electrodesorption has been mostly carried out in connection with zone electrophoresis[3,4] (see also ref. 2–11 cited in ref. 5). The disadvantage of this method is that the desorbed substance is obtained in a dilute state and its detection or isolation is complicated. Electrodesorption in connection with isoelectric focusing[6,7] concentrates a desorbed substance but it is limited to amphoteric substances with isoelectric points in the pH range attainable by isoelectric focusing. There is a risk of precipitation of desorbed protein at its isoelectric point and the desorbed substance may be contaminated with carrier ampholytes.

Electrodesorption in connection with isotachophoresis (ITP) regimen can be performed under conditions (pH, ionic strength) such that the chemical stability, solubility and biological activity of a desorbed substance is retained and owing to the concentrating and self-sharpening effect of ITP, the desorbed substance can be obtained in a concentrated state, contaminated only with the counter ion of the leading electrolyte. ITP electrodesorption has been used for the desorption of monoclonal antibodies against transferrin from the Insol–transferrin sorbent[5] and the above-mentioned advantages were verified in practice. ITP electrodesorption was performed in a capillary ITP apparatus equipped with a sorption element containing an affinity sorbent. The electrodesorption is connected on-line with quantitative ITP analysis of the desorbed substance and in this way quantitative evaluation of the sorption and desorption conditions on the micro-scale can be performed.

EXPERIMENTAL

Chemicals

Triton X-100, SPAN 80, Tween 20 and Tween 80 were obtained from Koch-Light (Colnbrook, U.K.), Brij 35 from Lachema (Brno, Czechoslovakia) and polyethylene glycols from Serva (Heidelberg, F.R.G.). Other chemicals were of the same origin as in ref. 5.

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Instrumentation and procedure

Desorption ITP was performed in the apparatus of our own construction[5]. A diagram of the apparatus used and its arrangement for the sorption and desorption processes and a scheme of the sorption element (type D was used) were given in a previous paper[5], where the procedure is also described.

RESULTS AND DISCUSSION

ITP electrodesorption of mouse monoclonal antibodies against porcine transferrin (MMAT) from the Insol–transferrin sorbent was performed under different sorption and desorption conditions and some important parameters of the process were tested.

Quantitative evaluation of sorption and desorption conditions is based on quantitative ITP analysis of the desorbed ligate. The amount of desorbed antibody m_D was determined by comparing its zone length l_D (corrected with respect to different electroosmotic flows in the presence of the sorption element in the capillary ITP apparatus) with the zone length l_S of a known amount of standard antibody m_S:

$$m_D = m_S l_D / l_S \quad (1)$$

The corrected zone length l_D is calculated from the measured zone length l_{DM}:

$$l_D = l_{DM} v_D / v_A \quad (2)$$

where v_D is the migration velocity of the zone of desorbed antibody in the presence of the sorption element and v_A is the migration velocity of the zone of the sample standard antibody. The migration velocities v_D and v_A are determined as the ratio of the known distance s (15 mm in our apparatus) between the potential gradient detector and the UV detector and the times t_D and t_A, respectively, during which the front boundary of the zone migrates through this distance at the same constant electric current (20 μA during detection in our case):

$$v_D = s/t_D \quad (3)$$

$$v_A = s/t_A \quad (4)$$

TABLE I

COMPOSITION OF ELECTROLYTE SYSTEMS FOR ITP DESORPTION OF MMAT

BALA = β-alanine; EACA = ε-aminocaproic acid; MES = 2-(N-morpholino)ethanesulphonic acid; PVA = poly(vinyl alcohol) (Mowiol).

System No.	*Leading electrolyte*				*Terminating electrolyte*		
	Leading ion (mol l^{-1})	*Counter ion*	*pH*	*Additive (%, w/v)*	*Terminating constituent (mol l^{-1})*	*Counter ion*	*pH*
I	K^+ 0.01	CH_3COO^-	5.2	PVA 0.02	BALA 0.01	CH_3COO^-	5.0
II	K^+ 0.01	MES^-	5.9	–	EACA 0.01	Cl^-	5.2
III	K^+ 0.005	MES^-	5.7	PVA 0.01	EACA 0.01	Cl^-	5.2

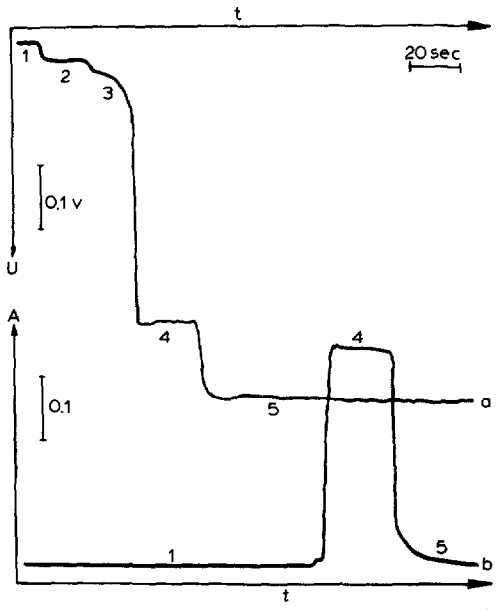

Fig. 1. ITP analysis of standard antibody MMAT in electrolyte system III (see Table I). Sample, 5 μl of a 1% (w/v) solution of MMAT in PBS; current, 50 μA (20 μA during detection); temperature, 20°C. 1 = Na^+; 2, 3 = unidentified admixtures; 4 = MMAT; 5 = EACA. a, Potential gradient detector signal; b, UV detector signal; *A*, absorbance; *U*, voltage of PG detector; *t*, time.

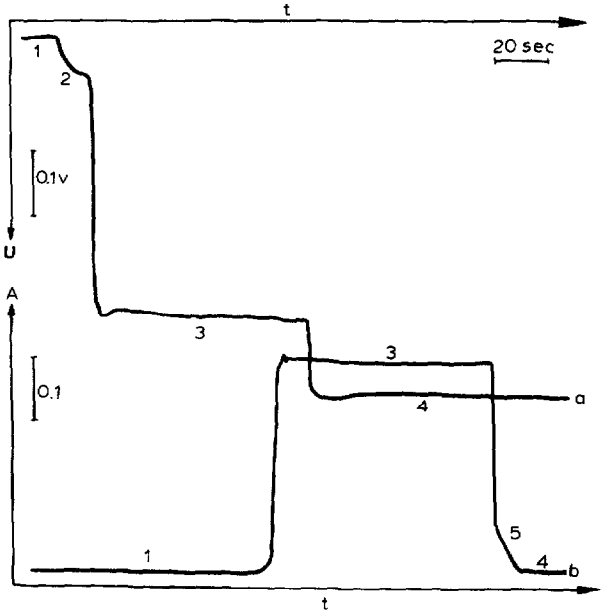

Fig. 2. ITP analysis of on-line desorbed MMAT in electrolyte system III (see Table I). Sample, 20 μl of a 1% (w/v) solution of MMAT in PBS made up to 1 ml with leading electrolyte sorbed at a flow-rate of 4 ml h^{-1}, washed with 0.5 ml of leading electrolyte, at the same flow-rate. The sorbent (10 μl) was treated with 0.1% PEG 20,000 (see Table V); desorption time 65 min; current, 50 μA during desorption, 20 μA during detection. a, PG detector signal; b, UV detector signal. 1 = Na^+; 2, 5 = unidentified admixtures; 3 = MMAT; 4 = EACA. *A*, absorbance; *U*, voltage of PG detector; *t*, time.

TABLE II

ITP ELECTRODESORPTION OF MMAT IN DIFFERENT ELECTROLYTE SYSTEMS

Values in parentheses are standard deviations calculated from 3–5 measurements.

Electrolyte system	*Amount of MMAT applied (μg)*	*Amount of MMAT desorbed (μg)*	*Activity of desorbed MMAT (%)*	*Steady-state concentration of MMAT (%, w/v)*
I	200	66.7 (2.7)	55.8 (2.3)	3.2
II	200	61.7 (2.5)	70.7 (3.2)	4.0
III	200	62.0 (2.7)	68.6 (3.5)	2.6

The amount of desorbed antibody was determined in this way in different electrolyte systems (see Table I). With respect to the isoelectric points, solubility and biological activity of given monoclonal antibodies[8], a cationic regimen was used. ITP analysis of standard antibody and desorbed antibody is shown in Figs. 1 and 2.

The amount of desorbed antibody in different electrolyte systems (see Table II) does not depend significantly on pH and ionic strength in the tested range, but the binding (immunochemical) activity of antibodies desorbed in the electrolyte systems II and III is about 15% higher than the activity in system I (see Table II). This can be explained by the fact that the pH of the terminating electrolyte of system I in the steady state is decreased to about 4, and at this value the immunochemical activity of the desorbed antibody can be partially destroyed[9], whereas the pH of the terminating electrolyte of systems II and III in the steady state is about 5 and at this value the activity of antibody is retained. The pH of the terminating electrolyte in the steady state was calculated using a program for the calculation of important parameters of the ITP steady state[10].

Because there was no possibility of isolating the desorbed MMAT from our analytical apparatus, the immunochemical activity was tested in such a way that the desorbed MMAT was returned by a hydrodynamic flow of leading electrolyte into the sorption element, where the active part was sorbed again and then a new electrodesorption was performed. The amount of desorbed MMAT in this second electrodesorption process is taken as a criterion of immunochemical activity. High values of the activity of desorbed MMAT confirm the advantage of the non-destructive conditions of ITP electrodesorption. The concentrating effect of ITP is demonstrated

TABLE III

ITP ELECTRODESORPTION OF MMAT AT DIFFERENT FLOW-RATES DURING SORPTION

Values in parentheses are standard deviations calculated from 3 measurements.

Peristaltic pump volumetric flow-rate ($ml\ h^{-1}$)	*Adsorption element linear flow-rate ($ml\ cm^{-2}\ h^{-1}$)*	*Adsorption time (min)*	*Amount of protein applied (μg)*	*Amount of protein desorbed (μg)*
2	27.8	45	200	70.0 (3.3)
4	55.6	22.5	200	68.4 (2.8)
8	111.2	11.8	200	65.0 (2.9)
16	222.4	5.9	200	27.6 (2.3)

TABLE IV

MASS BALANCE OF THE SORPTION AND DESORPTION PROCESSES

Values in parentheses are standard deviations calculated from 4 measurements.

Amount of protein	*%*
Applied (200 μg)	100
Unsorbed	35.7 (6.2)
Washed out	15.7 (3.7)
ITP desorbed	38.5 (3.3)
Losses	10.1 (6.8)

in Table II, where the steady-state concentrations of desorbed MMAT are given. The concentrating factor is 130–200 [the protein concentration before sorption was 0.02% (w/v)].

The amount of desorbed MMAT was also determined for different flow-rates during sorption. The results (see Table III) show that the antigen–antibody complex is kinetically labile and is formed even at relatively high linear flow-rates. Kinetic lability is important for fast and efficient electrodesorption.

The capacity of the sorbent was determined as the amount of desorbed MMAT of a fully saturated sorbent. Owing to the sorbent treatment with surfactants, the capacity was increased to 13 $\mu g\ \mu l^{-1}$.

The amount of unsorbed and washed protein in the sample solution after its passage through the adsorption element was determined colorimetrically according to Lowry *et al.*[11]. The mass balance of the sorption and desorption processes is given

TABLE V

INFLUENCE OF SORBENT TREATMENT WITH DIFFERENT SURFACTANTS ON THE AMOUNT OF DESORBED MMAT AND ITS ACTIVITY AFTER ELECTRODESORPTION

PEG = polyethylene glycol. Values in parentheses are standard deviations calculated from 3 measurements.

Surfactant	*Relative amount of MMAT desorbed (%)*	*Activity of desorbed MMAT (%)*
None	16.7 (3.5)	68.6 (5.1)
Triton X-100	6.7 (2.5)	41.1 (3.4)
Span 80	15.2 (2.5)	34.0 (2.5)
Tween 80	16.2 (2.1)	89.0 (4.3)
Brij 35	25.0 (4.5)	72.2 (5.0)
Tween 20	36.2 (2.5)	85.6 (2.3)
PEG 200	52.8 (4.7)	68.7 (5.1)
400	52.4 (4.0)	75.8 (5.7)
750	47.0 (4.6)	72.8 (4.8)
1500	52.0 (3.2)	67.2 (2.5)
6000	51.2 (3.7)	69.6 (5.1)
20,000	63.4 (1.2)	75.8 (3.6)
40,000	50.6 (4.5)	56.7 (5.2)

in Table IV. The losses (in some instances up to 20–25%) are caused by irreversible adsorption and by experimental errors with the methods used.

In order to suppress non-specific sorption, different surfactants were used for treating the sorbent before or during sorption. The sorbent was treated with 1 ml of 0.1% (w/v) solution of surfactant in the leading electrolyte at a flow-rate of 4 ml h^{-1}. A significant influence of sorbent treatment with surfactants on the amount of desorbed MMAT and its activity was found (see Table V). A positive influence of the polyoxyethylene chain of the surfactants used on the yield of active antibody was discovered. Other parts of the surfactant molecule, especially *p*-octylphenol in Triton X-100, decrease the yield of active antibody. The positive influence of surfactants can be explained by the fact that they preferably occupy sites on the sorbent where irreversible or non-specific sorption occurs. Further investigations must be made to explain the role of surfactants in more detail.

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