ELECTROPHORESIS: A NEW PREPARATIVE DESORPTION TECHNIQUE IN AFFINITY CHROMATOGRAPHY (AND IMMUNOADSORPTION)

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1. Introduction

A wide variety of methods exists for the elution of adsorbed material from affinity matrices which depend in many instances upon the nature of the interactions involved. For example, human serum albumin (HSA) can be eluted from Cibacron blue F3G-A–Sepharose using sodium thiocyanate, but the resulting HSA may not be clinically acceptable [1]. Likewise, ferritin can be removed from immobilised ferritin antibody columns by elution with 6 M guanidine–HCl (pH 3.1), but the eluted protein is unstable and precipitates on standing [2].

In spite of these disadvantages, chaotropic reagents are frequently used to overcome such high affinity interactions. The adsorption of antibody to immobilised steroid is such that conventional elution methods have proved less than satisfactory. Buffers of pH 3.0, containing 10% dioxane were used [3] to elute testosterone antibodies from an affinity column, and oestradiol-6-(*O*-carboxymethyl)oxime [4] to displace oestradiol antibody from complexes with immobilised steroid. In both cases the recovery of high affinity antibodies was poor.

The combination of electrophoresis and bio-specific interactions has been exploited analytically. The kinetics of the interaction of glycogen with various rabbit tissue phosphorylases [5], and of glycogen with potato phosphorylase and starch in human salivary amylase [6], have been studied by this method. Affinity electrophoresis has been used to investigate the interactions occurring in simple biospecific adsorption systems [7–11] and the conditions for such analytical applications have been optimised [12].

This technique could be extended [13] to the preparative elution of material from affinity matrices. Thus ferritin has been eluted from immobilised ferritin antibody [2] and competent steroid antibodies have been obtained in high yield from immobilised steroid [14–16] using the electrophoretic technique. This finding has been confirmed [17]. This electrophoretic desorption method avoids chaotropic conditions, and is one that will overcome even the strong interactions of haptens for immunoadsorbents.

We report here the electrophoretic desorption of HSA from Cibacron blue F3G-A–Sepharose, and of ferritin and oestriol-16α-glucuronide from the appropriate immobilised antibodies. We have studied the patterns of elution, and carried out experiments in such a way that the results enable us to predict a widespread applicability for the technique with high yields for the desorption of materials from affinity columns.

2. Materials and methods

2.1. *Preparation of the affinity matrices*

Sepharose was obtained from Pharmacia, London W5. Cibacron blue F3G-A (as the free triazine; see [18]) was a gift from Ciba-Geigy, Manchester. Cibacron blue F3G-A–Sepharose was prepared as in [19]. Adsorption of HSA was affected with 0.5 mg HSA/ml 0.08 M Tris/glycine buffer (pH 8.6) and followed by A_{280} measurement. When the absorbance of the eluted fractions reached that of the applied solution, the column was washed with the same buffer until the A_{280} declined to zero.

Anti-ferritin antibody was prepared and coupled to Sepharose 4B as in [2]. Serum (IL) was incubated with the immunoadsorbent for 2 h at 4°C with continuous mixing. The charged affinity matrix was then filtered, packed into a small (1 × 20 cm) column, and washed with 0.01 M sodium phosphate-buffered saline, at pH 7.4, until the wash A_{280} declined to zero.

Anti-oestriol-16α-glucuronide antibody was prepared and coupled to Sepharose 4B as in [16]. Tritiated steroid [6,9(*n*)-^{3}H]oestriol-16α-(β-D-glucuronide), spec. act. 30 Ci/mmol was from the Radiochemical Centre, Amersham. Typically, 100 000 dpm steroid was mixed at 4°C for 15 h with 0.1 g affinity matrix. The matrix was then washed with 8 × 5 ml portions of the 0.08 M Tris/glycine buffer (pH 8.6). The tritium content of the washes (determined using a Nuclear Chicago Isocap 300 Scintillation counter with Scintillation Fluid (NE 260) from B.D.H., Poole: tritium-counting efficiency 48) reached a constant level at 500 dpm after 5 washes.

2.2. *Electrophoretic desorption of the charged affinity matrices*

Gel electrophoresis was performed on 7% polyacrylamide gels at room temperature as in [20]. The gels were 4 cm long with an 0.5 cm diameter and subjected to 2 mA/gel, unless otherwise stated. Tris/glycine buffer (0.08 M, pH 8.6) was used in the upper and lower reservoirs in all instances. The charged affinity matrices were placed on top of the gels after pre-electrophoresis. The electrodes were arranged so that negatively-charged ions would move through the gels to the anode. All relative mobilities were calculated relative to a tracker dye, bromophenol blue.

2.3. *Assay of the gels after electrophoresis*

After the electrophoretic desorption of HSA from Cibacron blue F3G-A–Sepharose, gels were stained in 1% napthalene black in 7% acetic acid for 45 min and then destained in 7% acetic acid for 15 h. The gels were then photographed, and scanned at 450 nm in a Gilford 250 gel scanner.

After the electrophoretic desorption of ferritin from immobilised anti-ferritin antibody the gels were stained for iron with potassium ferrocyanide and for protein with Coomassie blue. The gels were then photographed and scanned as before.

After the electrophoretic desorption of steroid from immobilised oestriol-16α-glucuronide antibody, the gels were cut into 2 mm sections and each section stood in 1 ml water for 15 h. Scintillation fluid (4 ml) was then added and the tritium content determined.

3. Results

3.1. *Desorption of HSA from Cibacron blue F3G-A–Sepharose*

Figure 1 shows the pattern of desorption of the HSA from the affinity support into the polyacrylamide gel. Free HSA not mixed with affinity matrix has the same relative mobility in this system as the front of the desorbed HSA, and runs as a narrow band.

Increasing the time between the washing of the matrix and the electrophoretic desorption resulted in an increase in the initial peak height.

Figure 2 shows the effect of increasing the surface area of the gel in contact with the affinity support. The results of desorbing into a 0.8 cm diam. gel are compared to those obtained with a 2.5 cm diameter gel. The current/unit cross-sectional area was the same in both cases, as was the amount of material being desorbed.

The results show that the relative mobilities of the HSA front are the same in both instances and

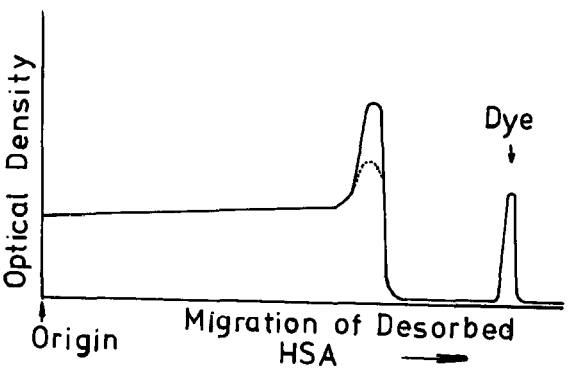

Fig.1. The pattern of desorption of HSA from Cibacron blue F3G-A–Sepharose as shown by the absorbance of the gel after staining for protein. The dashed line (– – –) shows the effect on the pattern of decreasing the time between the washing of the affinity matrix and beginning the desorption.

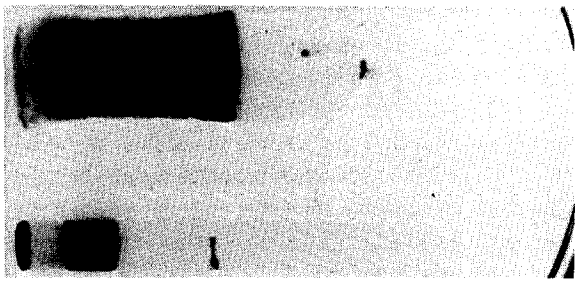

Fig.2. The effect of increasing the surface area of contact between the affinity matrix and the gel upon the electrophoretic desorption of HSA from Cibacron blue FG3-A–Sepharose. The HSA unbound at the start of the desorption is the same in both cases. The desorption that follows takes place at a much faster rate in the gel of larger diameter. For experimental details see text.

are equal to that of free HSA in the absence of the affinity matrices. The amount of HSA in the initial peaks of desorption is the same in both cases, and so the only difference between the two desorptions is the succeeding trail of HSA, which contained more concentrated protein in the gel providing the greatest surface contact with the affinity matrix.

3.2. *Desorption of ferritin from immobilised ferritin antibody*

Figure 3 shows the pattern of desorption of the protein ferritin from the immobilised ferritin antibody

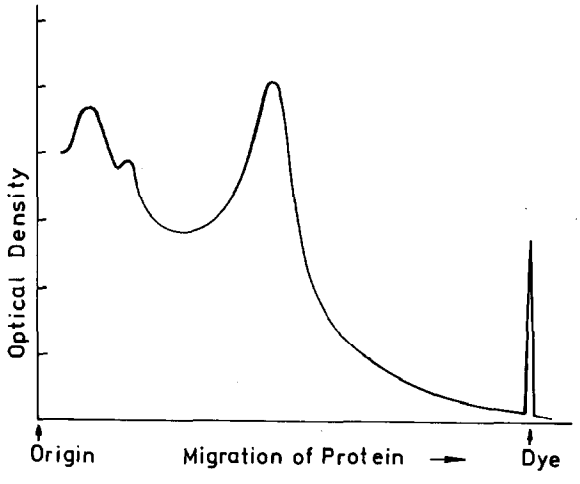

Fig.3. The pattern of desorption of ferritin from anti-ferritin antibody–Sepharose as measured by the absorbance of the gel after staining for protein.

column. Although the presence of polymeric forms of the ferritin are a complication it seems that the pattern is the same as that for the HSA desorption, i.e., an initial peak amount followed by a trail which decreases as the amount of material remaining to be desorbed decreases.

3.3. *Desorption of oestriol-16α-glucuronide from immobilised oestriol-16α-glucuronide antibody*

Figure 4 shows the effect of increasing the ratio of immobilised antibody to steroid. After incubating the immobilised antibody with steroid, equal portions were placed on top of the polyacrylamide gels as described. Known amounts of immobilised antibody (without steroid) were then added to give various ratios of steroid to immobilised antibody. Sepharose was added where necessary to ensure that the height of the beads on the gels was the same in each case. After mixing, electrophoretic desorption was carried out.

It can be seen that though the amount of steroid present in each case is the same, the desorption pattern is different. As the ratio of antibody to steroid increases, both the initial peak height and the subsequent trailing decrease.

4. Discussion

The patterns of desorption reported here for HSA from Cibacron blue F3G-A–Sepharose, for ferritin from anti-ferritin antibody–Sepharose, and for oestriol-16α-glucuronide from anti-oestriol-16α-glucuronide antibody–Sepharose are the same, and appear to be typical of all electrophoretic desorptions. Another example is the desorption of oestriol-16α-glucuronide antibody from immobilised steroid columns [4].

These patterns take the form of an initial peak of desorbed material, which is followed by a 'trail' that decreases until no more material remains on the affinity matrix.

The amount of material represented by the height of the initial peak is related to the time between the washing of the affinity matrices and the beginning of the desorption.

The relative mobilities (as measured by the position of the initial peak) of compounds electropho-

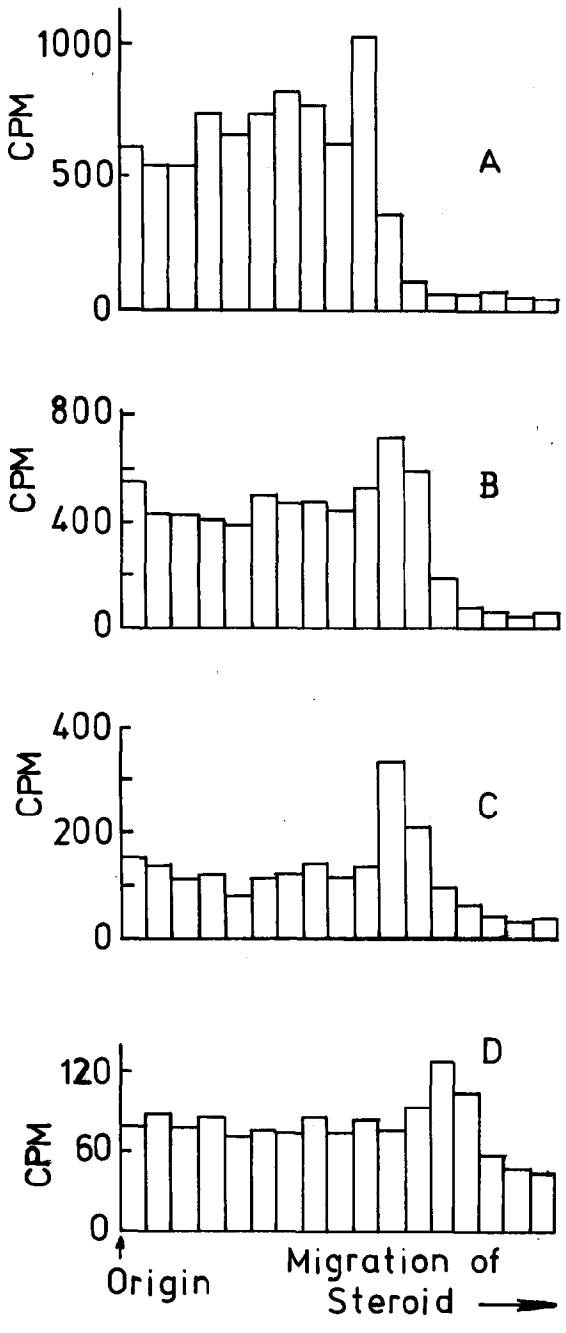

Fig.4. Tritium cpm in 2 mm gel slices after electrophoretic desorption of tritiated oestriol-16α-glucuronide from immobilised antibody matrix. The amounts of steroid and Sepharose (substituted and unsubstituted) is the same in all cases. The ratio of antibody–Sepharose to steroid in (A) is doubled in (B), increased 5 times in (C) and increased 10 times in (D). The average background counting value is 45 cpm.

retically desorbed from affinity matrices are those obtained for the compounds in the absence of any affinity matrix. Thus the desorption process begins immediately the current is applied.

We propose that the mechanism of desorption is the same in all the instances reported here. The observed initial peak represents material which is unbound at the start of the desorption. This material has dissociated from the ligand after the washing procedure has removed the excess, free material. The amount of material observed in this peak is both time- and K_a-dependent. The 'trail' that follows the unbound material into the gel is that which dissociates and migrates continuously in the ensuing period. This dissociation persists until no material remains on the support. The desorption is therefore a passive procedure. Ions will move in the field towards the gel only when dissociated; if they re-associate with immobilised ligand then their migration rate may be proportionally retarded. We would predict therefore that the rate at which a material can be desorbed will be directly related to the rate of dissociation for that material and the immobilised ligand.

In the experiment where the desorption of HSA on two gels of different diameters is compared under identical conditions, it is seen that the greater the surface area between the gels and affinity support the faster is the rate of desorption. This is because the increased surface area increases the probability of migration into the gel without re-association occurring since the distance the ions have to migrate is less than with a smaller surface area in contact with the gel. Thus the rate of desorption with narrow gels is slower than for wide gels for the same amount of affinity matrix.

Similarly, the results of the experiment where the ratio of immobilized antibody to steroid was altered can be explained by the greater probability of re-association of the steroid with the affinity support when the amount of immobilised antibody is increased. In this instance, the steroid is desorbed at a much slower rate.

In accordance with the postulated mechanism, factors affecting the binding of the material to the affinity support, such as changes in pH, or changes in temperature will also influence the observed rates of desorption. Any compound adsorbed non-covalently by an affinity (or other) interaction to an immobilised

support should be able to be desorbed by this electrophoretic method, provided that:

(i) The interaction is reversible.

(ii) The compound to be desorbed is charged.

The electrophoretic desorption technique used [17] to remove steroid-specific antibodies from immobilised steroid columns, failed to obtain antibodies of high affinity. Our proposed mechanism suggests that an increase in the time of desorption would be necessary in order to obtain these antibodies. If the time required proved excessive, then a change in the surface area of contact between the affinity matrix and the gel would be beneficial.

One of the few affinity interactions in current use which does not fulfill both the above conditions is that of sugar and immobilised lectin. However, lectins could be electrophoretically desorbed from immobilised sugars. Therefore this essentially non-chaotropic method would appear to be widely applicable, especially in the field of immunoadsorbents where conventional elution techniques are often unsatisfactory.

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