ELECTROPHORESIS OF LIGANDS OVER A SURFACE COATED WITH A BINDING RECEPTOR

A novel methodological principle for electroimmunoassays

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1. Introduction

Immunological methods based on electrophoretic movement of reactants in a gel have been widely exploited during the last decades [1–3]. Quantitative immunoelectrophoretic methods are based on the electrophoresis of antigen into an antibody-containing gel causing a specific immuno-precipitation of the antigen. The area enclosed by this precipitate is quantitatively related to the concentration of antigen and antibody in the system. The applicability of the method is limited by the electrophoresis conditions which have to be such that the antibodies do not move in the electric field.

A new approach has been taken to the quantitation of antigen and antibodies by diffusion of one reactant over a surface, coated with the other reactant [4–7]. The quantitation of diffusing ligand in these methods is based on its ability to form a concentration gradient over the binder-coated surface, and the visualisation of the reaction could be performed by several techniques, e.g., hemadsorption [4], water condensation [5], enzyme-labelled antibody [6] or ellipsometric analysis of the surface [8]. The advantage of these methods is that they are simple to perform, sensitive and accurate. The disadvantage is that the diffusion is a slow process which makes the methods time-consuming.

This study describes the electrophoresis of ligands over a binder-coated surface as a novel methodological principle and presents the use of this principle for quantitation of antigen, antibodies and for quantitation of cholera toxin based on its binding to the ganglioside GM_1. The sensitivity of the novel method was 0.1 mg/l of specific antibodies and cholera toxin and 0.4 mg/l of antigen. The electrophoresis conditions could be chosen freely, as the binder was immobilised on the surface. The surface reaction was saturated in <6 h, which is <10% of the time necessary for the surface reaction to reach saturation during diffusion.

2. Materials and methods

2.1. *Antigen and antibodies*

Bovine serum albumin (BSA) (Sigma Chemical Co., St Louis MO) and anti-BSA were used as model system. Anti-BSA serum was a gift from Dr L.-Å. Nilsson, Institute of Medical Microbiology, University of Göteborg.

For some experiments, antibodies were isolated by immunosorbent technique, using BSA immobilised to CNBR–Sepharose 4B (Pharmacia Fine Chemicals, Uppsala) as immunosorbent.

2.2. *Cholera toxin and ganglioside*

Pure and homogenous preparations of cholera toxin and ganglioside GM_1 were gifts from Drs Stefan Lange and Ivar Lönnroth, Dept. of Bacteriology, Institute of Medical Microbiology, Göteborg.

2.3. *Conjugates*

The immunoglobulin fraction of anti-BSA antiserum was coupled to horseradish peroxidase as in [9]. *Staphylococcus* protein A was coupled to peroxidase as in [10].

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2.4. *Agarose and buffers*

Agarose (type A,B or C) was obtained from Pharmacia Fine Chemicals, Uppsala. The agarose (2 g/100 ml) was melted in distilled water and mixed with an equal volume of pre-warmed 0.1 M veronal buffer (pH 8.6 or 6.5), 0.05 M borate–HCl buffer (pH 8.9), 0.05 M phosphate buffer (pH 6.0 or 6.5), or 0.1 M glycine–NaOH buffer (pH 9.5).

2.5. *General methodological performance*

The methodological principle is shown in fig.1. The experiments were performed as follows: The inside of polystyrene petri dishes were coated with BSA (0.1 g/l in saline for 16 h), ganglioside GM_1 (3 ng/l in saline for 16 h with subsequent coating with BSA for 2 h) or antibodies isolated by immunosorbent (0.1 g/l, 16 h). Agarose solution was poured over the plates and was allowed to settle.

Basins (3 mm diam.) were punched out in the gel, and the plates were placed in a conventional flat bed electrophoresis apparatus. Samples (5 μl) were applied into the basins and electrophoresis (2–5 V/cm) was run towards the anode or towards the cathode for 4–20 h, as indicated in the figures. The gel was removed after the electrophoresis, and the receptor–ligand reaction zones were visualised by water condensation [5] or by enzyme-labelled probe [6].

3. Results

3.1. *Quantitation of antibodies by electrophoresis over an antigen-coated surface*

Peak-formed antigen–antibody reaction zones could be detected on the surface by WCS (fig.2a) and ELISA visualisation systems (fig.2b). The length of the reaction zones increased up to an electrophoresis time of ~18 h. Dilutions of the antiserum showed that there was a quantitative relation between the amount of antibody and the length of the reaction zone. The sensitivity of the method in its present form was 0.1 mg/l.

3.2. *Quantitation of antigen by electrophoresis over an antibody-coated surface*

Peak-formed antigen–antibody reaction zones could be detected on the surface by ELISA detection (fig.3) using peroxidase-labelled antibodies of the same specificity as those immobilised on the surface. The length of the reaction zones increased for up to 6 h for diluted samples. There was a quantitative relationship between the amount of antigen and the length of the reaction zones. The sensitivity of the method for detection of antigen was 0.3 mg/l.

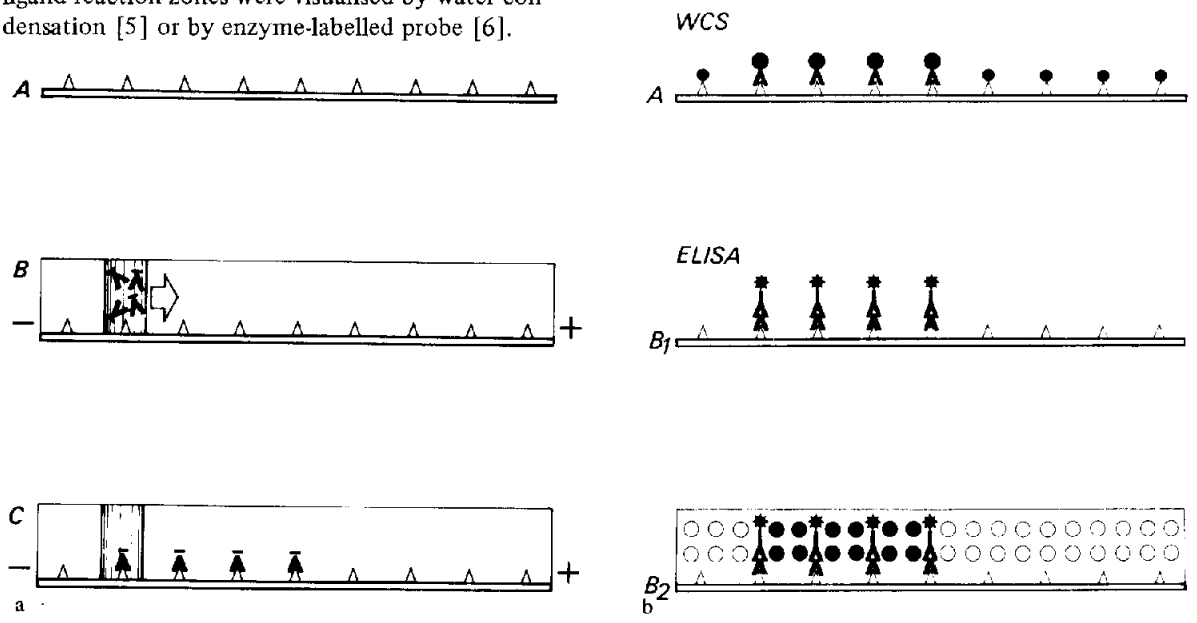

Fig.1. The principle of the electrophoresis of ligands over a surface coated with a binding receptor. (a) Quantitation: (A) a hydrophobic surface is coated with the binder; (B) a gel is poured over the surface, and the ligand is pipetted into wells in the gel; (C) the ligand moves in the electric field and is bound to the binding receptor. (b) Visualisation: (A) water is condensed on the surface. A hydrophilic condensation pattern is formed over the binder–ligand reaction zones; (B_1) an enzyme-labelled antibody is bound to the binder–ligand complex on the surface; (B_2) the enzyme is visualised by a substrate reaction yielding a coloured reaction product.

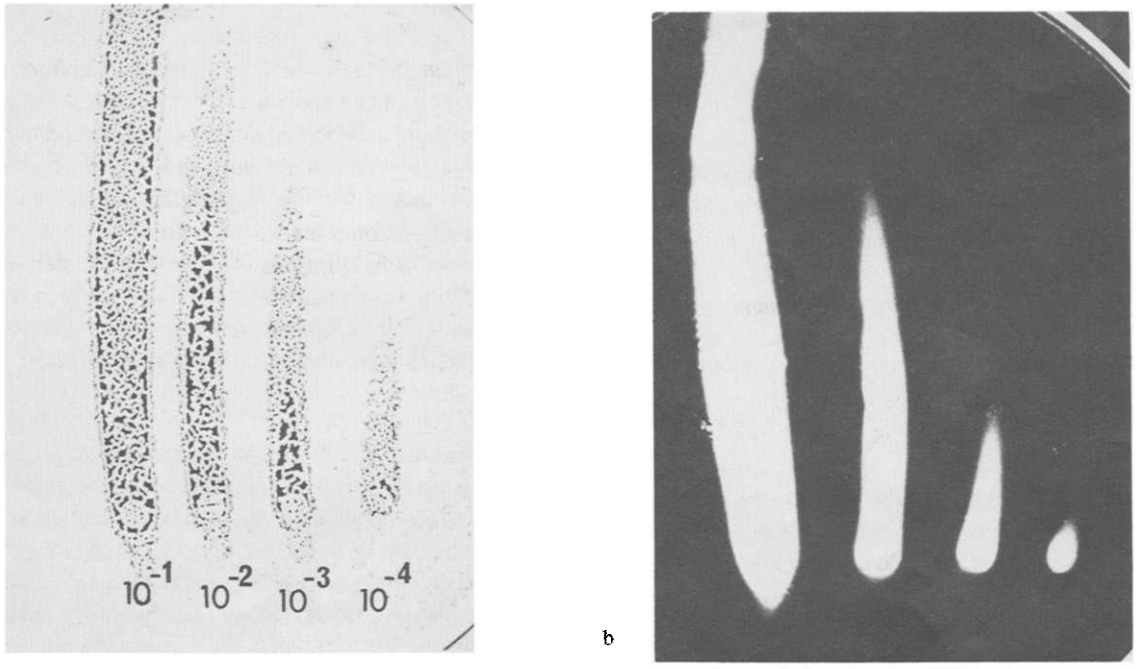

Fig.2. (a) Antigen–antibody reaction zones visualised by water condensation. The surface was coated with BSA. Anti-BSA antibodies (serum dilution 1:10–1:10 000) were electrophoresed against the cathode (top) for 16 h (phosphate buffer, pH 6.0). (b) Same as in (2a) but with ELISA visualisation procedure.

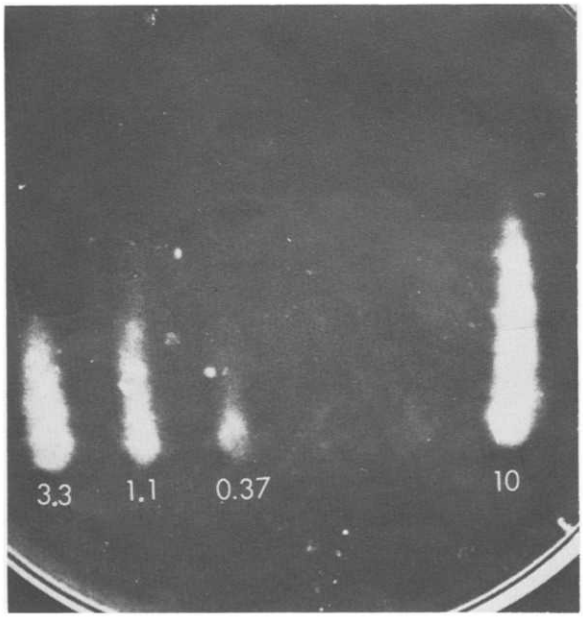

Fig.3. Antigen–antibody reaction zones visualised with ELISA visualisation procedure. The surface was coated with specific anti-BSA antibodies. BSA (10–0.37 mg/l) was electrophorised against the anode (top) for 4 h (phosphate buffer, pH 6.5).

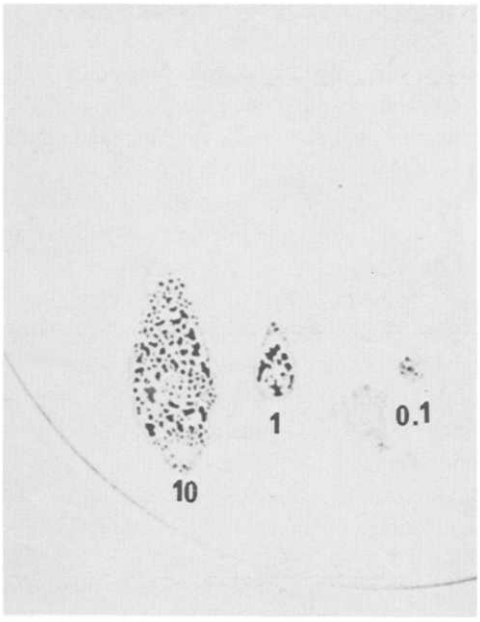

Fig.4. Cholera toxin–GM_1 reaction zones visualised with water-condensation. The surface was coated with GM_1. Cholera toxin (10–0.1 mg/l) was electrophorised against the anode (top) for 4 h.

3.3. *Quantitation of cholera toxin by electrophoresis over a ganglioside-coated surface*

Quantitation of cholera toxin was included into the experiments to demonstrate a general applicability of the method principle. Cholera toxin–ganglioside reaction zones could be detected by WCS (fig.4) or ELISA visualisation systems, and there was a quantitative relationship between the amount of toxin in the sample and the length of the reaction zones. The sensitivity of the method for detection of cholera toxin was 0.1 mg/l.

4. Discussion

The results of these experiments demonstrate the possibility to make a quantitative determination of ligands by electrophoresis over a binder-coated surface. By using this principle, it was possible to quantitate specific antibodies, antigen and a bacterial toxin. The quantitation of ligands is based on the visualisation of ligand–binder reaction zones on the surface. The growth of these zones with time is not only influenced by parameters determining the moving of the ligand in the electric field, but also by diffusion of the ligand vertically down to the surface and by the type of reaction taking place on the surface. Reversible or irreversible reactions, diffusion-rate limited or reaction-rate limited reactions will result in different dose–response profiles [11,12]. The surface reactions used as model systems here could be described as irreversible and diffusion-rate limited, and the sensitivity of the detection systems has been calculated to be 160 ng/cm^2 for WCS and 10 ng/cm^2 for ELISA [12].

The sensitivity of this method depends thus on the sensitivity of the visualisation system and on the surface concentration of ligand, which in turn is dependent on the surface density of the binder and the molecular size of the ligand. The sensitivity of the method in its present form is in the same magnitude as that of traditional 'rocket' electrophoresis. The main advantage of this method, compared to the traditional 'rocket' electrophoresis [13] for quantitation of antibodies is that in the former, one of the reactants is immobilised on the surface whereas in the latter, one of the reactants is dissolved in the gel, electrophoretically immobilized by the choice of pH and quality of the gel, but freely diffusing in the gel. The immobilisation of one of the reactants on a surface implies a freedom in choice of electrophoresis conditions. The electrophoresis of the ligand could be performed at any pH, still permitting binding to the immobilised binder, and in any direction. Thus the electrophoresis of antibodies could be performed towards the cathode at 6.0–6.5 in veronal, phosphate or borate buffers, as in these experiments or at pH 8.9–9.5 towards the anode with the same result.

The main advantage of electrophoresis of ligands over a binder-coated surface as compared to diffusion of ligands over a binder-coated surface as in DIG–TIA [5] and DIG–ELISA [6,7] is that the diffusion is much more time-consuming than the electrophoresis. Maximal resolution of DIG–ELISA and DIG–TIA is obtained after 72–240 h, depending on the system used, whereas the electrophoresis could be completed within 4–20 h, dependent on the electrophoresis conditions.

The principle of electrophoresis of ligands over a binder-coated surface could also be used in the second dimension electrophoresis of crossed immunoelectrophoresis or immuno-isoelectric focusing.

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