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Two-dimensional electrophoresis: agarose gel isotachophoresis followed by sodium dodecyl sulphate–polyacrylamide electrophoresis

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ABSTRACT

High resolution of proteins by gel isotachophoresis (ITP) has been achieved in previous work, and two-dimensional electrophoresis was applied in order to obtain further information on the proteins separated by ITP in agarose gels. Gradient or discontinuous buffer acrylamide gels in the presence of sodium dodecyl sulphate (SDS) and β-mercapthoethanol were used for the second electrophoresis. After two-dimensional ITP–SDS polyacrylamide gel electrophoresis (PAGE), several components were observed on the plates after either silver or Coomassie Brilliant Blue staining. The enhanced technique with SDS-PAGE revealed further heterogeneity among C3-complement proteins, with at least eight α- and eight β-chains of C3-complement. Two albumins spots were also observed after staining. The proposed technique provides a powerful addition to earlier methods for the separation of proteins.

INTRODUCTION

The most striking results with two-dimensional (2D) electrophoresis were obtained by O'Farrell [1] by combining isoelectric focusing (IEF) and sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE). However, the use of IEF is limited by precipitation or aggregation phenomena because the solubility of most proteins is lowest at their isoelectric points. In addition, the behaviour of some proteins *e.g.* serum albumin, which spread over a wide pH zone on focusing, limits the application of IEF for the separation of blood plasma proteins. To overcome these problems, ITP has been applied. The relative migration of plasma proteins in ITP is similar to that obtained in electrophoresis, and the resolution of the proteins achieved by ITP is comparable that given by IEF but the drawbacks mentioned are avoided [2].

The application of 2D ITP–SDS-PAGE followed by Coomassie Brilliant Blue (CBB) staining, silver staining and sequential immunostaining for the analysis of plasma proteins is described in this paper.

EXPERIMENTAL

Human plasma samples were supplied by the blood centre at the Karolinska Hospital. Agarose, ampholines, electrode strips and paper sample applicators were

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obtained from Pharmacia LKB Biotechnology (Bromma, Sweden), servalytes from Serva Feinbiochemica (Heidelberg, Germany), nitrocellulose membrane Immobilon from Millipore (Bedford, MA, U.S.A.), rabbit immunoglobulins (Igs) and horseradish peroxidase-conjugated swine antibodies against rabbit Igs from Dakopatts (Copenhagen, Denmark). All chemicals were of analytical-reagent grade.

Isotachophoresis in agarose gels

The procedure was basically as decribed earlier previously [1]. Isotachophoresis was run in flat-bed agarose gels (12 × 24 cm, 1 mm thick). The plasma proteins were electrophoresed towards the anode in 1% agarose IEF. Appropriate combinations of ampholytes and servalytes were used as spacers [3].

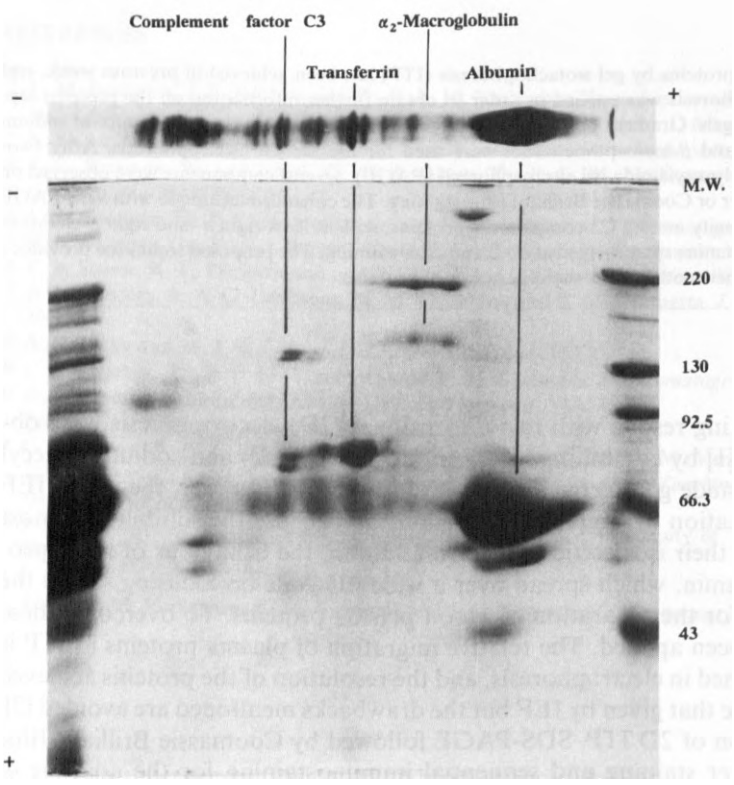

Fig. 1. 2D ITP–SDS-PAGE of human blood plasma proteins stained with CBB. First dimension (ITP) (top): leading ion, 40 m*M* glutamic acid; counter ion, 40 m*M* histidine; spacers, included in the gel, 1.2 ml of Servalytes 5–6; terminating ion; β-alanine; anode, 20 ml of 40 m*M* glutamic acid–250 m*M* histidine; cathode, 10 ml of 1 *M* β-alanine–250 m*M* histidine; power, 5W. Second dimension (discontinuous SDS-PAGE): separation gel, $T_{8.7}C_2$, in 400 m*M* Tris–HCl (pH 9.18); stacking gel, $T_{3.2}C_{6.25}$, in 125 m*M* Tris–HCl (pH 6.8); electrode buffer, 170 m*M* glycine–50 m*M* Tris. Left lane, human blood plasma sample; right lane, molecular weight standards. M.W. = molecular weights (kilodalton).

2D-Isotachophoresis–polyacrylamide gel electrophoresis

Strips 1.0 cm wide from the agarose gel plate were covered with buffer containing SDS and β-mercaptoethanol for 5 min. Thereafter, the strips were placed on the top of discontinuous 8.7% acrylamide gels [4] or 6–20% acrylamide gradient gels and run overnight. The proteins were revealed by CBB staining silver staining [5] or immunodetection [6,7].

Immunodetection

The transfer of the proteins to nitrocellulose (NC) was done by capillarity. Immunological detection was performed using peroxidase-labelled antibodies and staining with 4-chloro-1-napthol [7]. The NC filters were dried and photographed. If desired, the NC filters were used for the sequential immunological detection of other proteins.

RESULTS AND DISCUSSION

The relative positions of the non-denatured proteins in the ITP gels after the first dimension are determined by their electrophoretic mobilities, *i.e.*, α-, β- of γ-proteins. After denaturation with SDS and reduction with 2-mercaptoethanol, the sub-

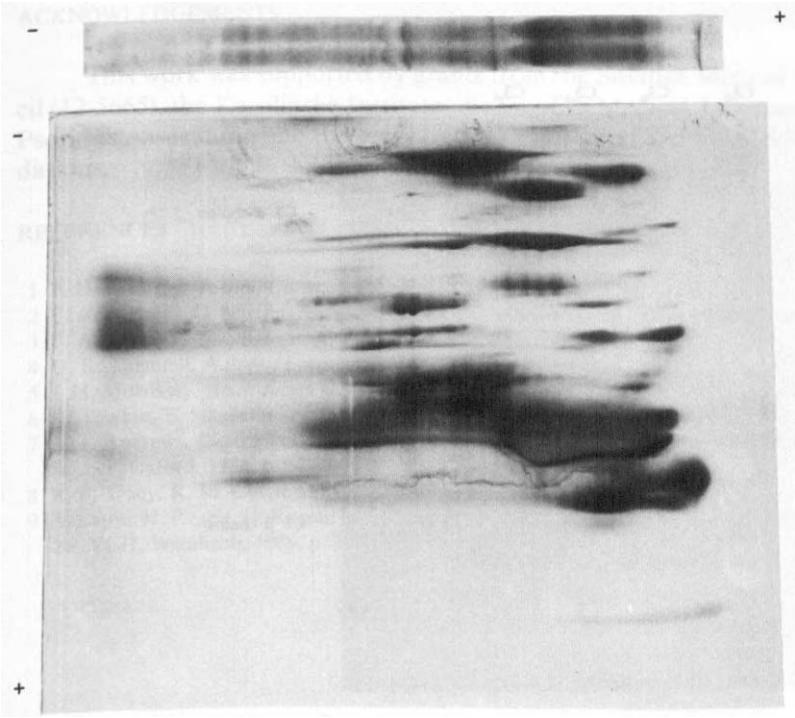

Fig. 2. Silver staining [5] in 2D ITP–SDS-PAGE of human blood plasma proteins. First dimension (ITP) (top): as in Fig. 1 except that the spacers were 0.8 ml of Ampholines 5–7 and 0.8 ml of Servalytes 5–6. Second dimension (gradient gel SDS-PAGE): gradient, T_6C_2 to $T_{20}C_2$ in 340 m*M* glycine–100 m*M* Tris buffer.

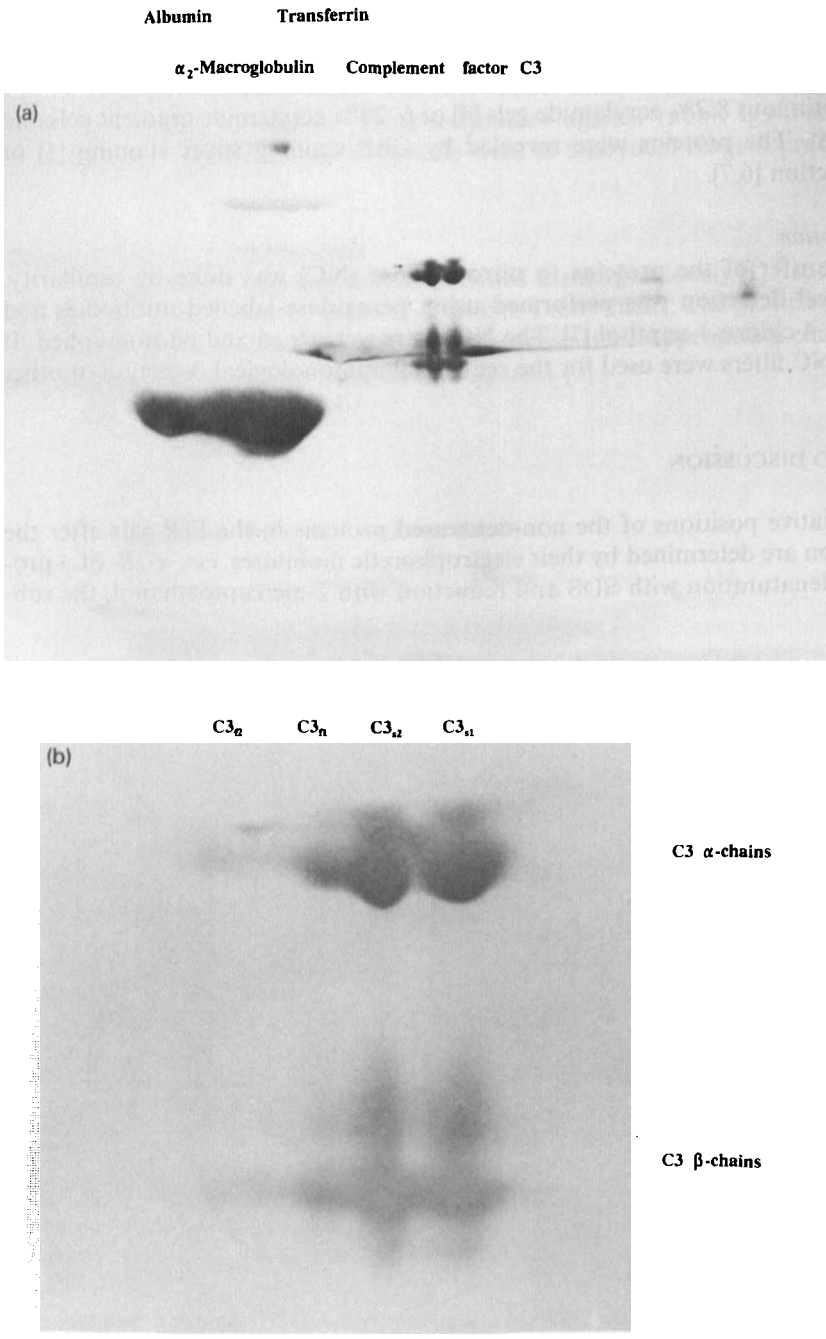

Fig. 3. Immunodetection of some human blood plasma components after 2D ITP–SDS-PAGE. (a) The stained proteins were albumin, α_2-macroglobulin, transferrin and C3-complement. (b) The α- and β-chains of C3-complement observed after 2D ITP–SDS-PAGE followed by immunostaining with antihuman $C3_c$.

units can be separated in acrylamide gels and revealed either with CBB, as in Fig. 1, with silver staining, as in Fig. 2 or by immunodetection, as in Fig. 3. After immunostaining with anti-human C3-complement at least eight α- and eight β-chains can be recognized (Fig. 3b). This heterogeneity has not been reported previously. At least two albumin spots can also be distinguished after immunostaining. These and other heterogeneities could also be observed after non-specific staining with CBB or silver.

The distribution of the proteins in the 2D ITP–SDS-PAGE differs from that obtained by 2D IEF–SDS-PAGE. Most of the stained compounds in the former show molecular weights over 40 kilodalton, whereas after 2D IEF–SDS-PAGE a high content of small proteins is observed [8]. This is due to the presence of denaturing agents required during the IEF separation of plasma proteins. The separation of human plasma proteins by 2D cellulose acetate–SDS-PAGE [9] produces a pattern resembling that obtained by 2D ITP–SDS-PAGE. However, the better resolution achieved by ITP permits the distinction of variants of several proteins.

The concentration step of the stacking zone in discontinuous buffer systems for SDS-PAGE, as in Fig. 1, causes a flattening of the sharp bands from the agarose migrating into the acrylamide gel with loss of resolution. Therefore, polyacrylamide gradient gels, as in Fig. 2, are more suitable for the second dimension in the technique presented here.

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