Characterization of transferrin subtypes by hybrid IEF on ultrathin polyacrylamide layers

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INTRODUCTION

Isoelectric focusing (IEF) on immobilized pH gradients (IPGs) is the method of choice for the characterization of a large variety of enzymes and seroproteins (Cleve et al. 1982; Görg et al. 1984a,b; Sutton and Westwood 1984). A vast array of theoretical and practical problems related to the application of IPGs have been by now solved, and well established protocols are available for any needed pH interval of isoelectric separation (Gianazza et al. 1985). However, in spite of the remarkable refinements of IPGs technology, a number of problems still hinder immobilized gradients to be used as a true routine procedure of electrophoresis. Noteworthy, the IPGs gel casting procedure is as much time-expensive as to discourage its universal use. IPGs are currently performed on polyacrylamide gels as thick as 0.5 mm, whose casting requires to assembly a repel-silanised glass plate with either a bind-silanised glass or a GelBond film, and a rubber spacer between. Silanisation happens to be one of the most tedious and time-wasting steps and, every now and then, even carefully silanised glasses do not prevent gels to tear out when opening the molds.
Another drawback is in the very long separation times required for ultranarrow pH intervals (< 1 pH unit).
For all these reasons, each IPGs experiment usually takes almost two days to completion.
Finally, immobiline chemicals are more expensive than commercial convective pH intervals.
We recently sought to circumvent these drawbacks, by resorting to an extremely simplified gel casting procedure, enabling to perform diluted (1.5 meqv/pH/L) IPGs on ultrathin slab gels, with considerable times and costs saving. The procedure is here described for the characterization of transferrin subtypes.

METHACRYLATE/GLASS MOLDS

The molding procedure is based on the use of a common (unsilanised) glass plate, assembled together with a

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polyacrylate- coated (120x 240 x 20mm) plate, with a very thin (0.25 mm) rubber frame between. Acryl-coated plates may be obtained by spraying common glass plates with a polyacrylic paint mixture (e.g. Standocryl 2K, Herberts GmbH, Wuppertal, FRG, diluted 1:4 with MS Verdunnung, Herberts, and supplemented with 50% w/v Standox 2K Harter 66755 Herberts). Acryl-coated plates provide a reusable, heat-resistant, highly-hydrophobic support, which is able to repel the polyacrylamide slab gels. The mold is filled from the top, and the polymerization is allowed to proceed as abitually (50°C for 1 h). After polymerization, when opening the molds, the acryl-coated glasses detach almost spontaneously, while the IPG gels firmly adher to the glass underneath by the interaction between polar immobiline radicals-silica dioxide of glass.

ULTRATHIN IPG SLABS

The acrylate/glass molds virtually allow to pour immobilized gradients in very thin gaskets (0.25 mm thick) since the capillarity generated between the two walls is very low. However, the amounts of dense and light solutions (3,5 ml each, on average) are too small for being evenly mixed by commercially available gradient mixers. For this reason, we used a home-made mixer with small capacity chambers. With this device, we poured a pH interval suitable for transferrin isoelectric point (pH 5.2-5.7, Immobiline amounts drawn by linear interpolation from recipes contained in LKB application note n° 324. Final concentration of immobiline chemicals was half the amount normally advised by the manufacturer (1.5 mEq/pH/L), and the gradient was supplemented with carrier ampholytes of proper range of pH (final CA concentration in gels: 0.5% v/v).

SAMPLE TREATMENT

Neuraminidase (Type VIII, Sigma, 5 U/ml in saline) treatment of serum (7 µl serum + 20 µl neuraminidase) was preferred to ferrous/ferric salts enrichment. Samples (20 µl on average) were soaked on filter paper rectangles and applied cathodally.

ELECTROPHORESIS

The application of ultrathin mixed beds IEF (Gelfi et al 1986), also called 'hybrid' IEF (Altland and Rossman 1985), allows to achieve well resolving separations in half the time normally required for normal-thickness IPGs. Transferrin subtyping was obtained by running gels for 20 KV/hs (initial settings: 200 V, 5 mA, 3.5 W, 10°C, final

voltage 5,000 V). In order not to burn out carrier ampholytes incorporated in the matrix, electrode wicks were employed (10 mM glutamic acid at the anode, NaOH 10 mM at the cathode). Focused gels were fixed in sulfosalycilic acid, then stained with Coomassie. Long stained gels tended to detach from the glass support at the anodal edge, due to the fact that the density medium used for the gradient (either glycerol or sucrose) was being washed away (this does no more occur if more acidic or basic pH intervals are cast). This phenomenon was simply counteracted by adding 5% glycerol to staining and destaining solutions. Stained gels were finally air dried on the same glass supports for permanent record.

CONCLUSIONS

The procedure above detailed allows a fine-quality resolution of transferrin subtypes, exemplified in Fig. 1. Its most outstanding improvements are: a) the molding procedure is fast and performed with wholly reusable materials; b) diluted IPGs in ultrathin-layers are by far less expensive than the traditional slabs; c) the times of separations are noticeably shortened (quite comparable to those required by convective IEF, if a high-voltage power supply is used);d) high-quality performances are obtained with lesser time and costs.
Needless to say, the procedure is largely applicable throughout the scale of pH covered by Immobiline chemicals, as well as for IPGs under denaturing conditions (Pascali et al. 1987).

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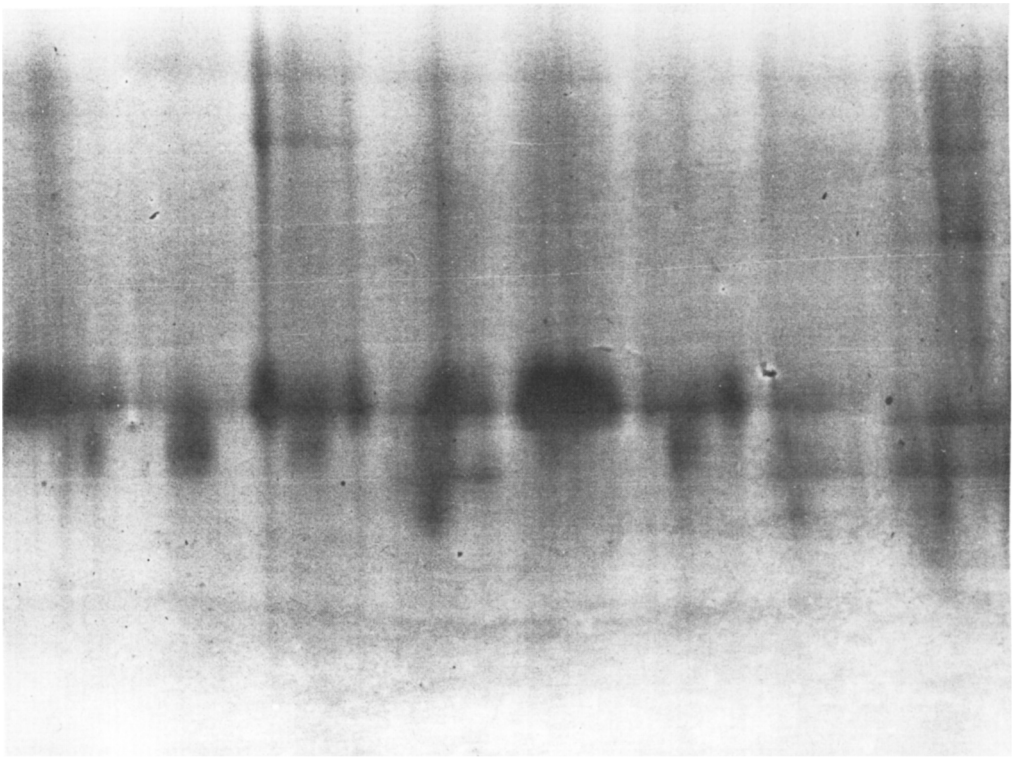

Fig. 1. Transferrin patterns after hybrid IEF on ultrathin layers. From left: C1, C1, C1, C2-1, C1, C1, C2-1, C3-2 (anode is on top).