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Note

Electrofocusing of stroma-free haemoglobin on a thin layer of agarose gel

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Electrofocusing of proteins on thin layer polyacrylamide gel (PAG) is a very useful and widely used method for detailed protein separations[1]. No electroendosmosis takes place in PEG so that the pH gradient formed during electrofocusing is stable. However, in addition to this important advantage, the use of PAG also has practical disadvantages, *e.g.*, slow and sometimes non-standard gel formation, difficult handling and storage, and toxicity of the components. We therefore attempted to use a thin layer of agarose gel instead of PAG. Our main interest was to establish whether isoelectric focusing could be achieved at all in this medium and how much the expected cathodic drift of the ampholyte interferes with the separation process. Haemoglobin served as a model substance.

MATERIAL AND METHODS

Agarose C, lot No. DA 2965 (Pharmacia, Uppsala, Sweden), was dissolved in distilled water at 90–95°C, the concentrations ranging from 10 to 15 g/l. A 1-ml volume of Pharmalyte (Pharmacia), pH range 5–8 or 3–10, was mixed thoroughly with each 14 ml of the hot agarose solution. The solution was poured on to a horizontal glass plate (85 × 85 × 1.5 mm) to form the gel. A Pharmacia FBE 3000 flat-bed apparatus and an ECPS 2000/300 power supply were used. Pre-focusing lasted 10–20 min, the voltage was between 25 and 65 V/cm and the power did not exceed 15 W. Stroma-free haemoglobin[2] from outdated human banked blood was stored for 6 months at −20°C before use. Desalted haemoglobin samples (about 20–50 μg of haemoglobin) were soaked into 5 × 5 mm filter-paper wicks and applied to the surface of the gel. Electrofocusing was performed in the usual manner[2] under the same conditions as were used during pre-focusing. Fixation of proteins, staining with Coomassie Blue G-250 and destaining were carried out as described earlier[2].

RESULTS AND DISCUSSION

Fig. 1 shows that electrofocusing of proteins can be performed with good results in the agarose gel. Separation of the haemoglobin fractions was generally similar to

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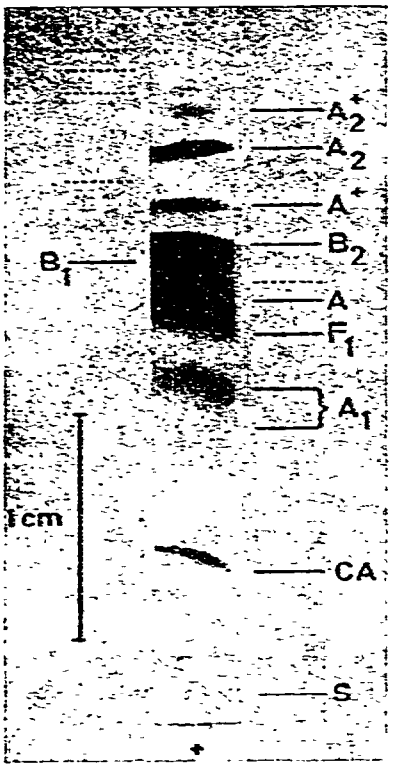

Fig. 1. Electrofocusing of human stroma-free haemoglobin in agarose gel. Agarose concentration: 10 g/l. Pharmalyte, pH 3–10. Pre-focusing at 43 V/cm for 20 min. Electrofocusing at 43 V/cm for 60 min. Staining with Coomassie Blue G-250. S = start; CA = carbonic anhydrase. A, B and F indicate haemoglobin fractions. Dotted lines indicate the positions of unidentified protein fractions.

that achieved in PAG[2]. However, the reproducibility of the results with agarose gel was not always satisfactory because of electroendosmosis.

Even during pre-focusing the gel began to change markedly along the anode. The electrolyte driven towards the cathode could not be absorbed by the rigid gel and was left on the surface as a separate fluid phase, which advanced irregularly towards the cathode. The excess of fluid had to be soaked off carefully before placing the samples at the start. A significantly dehydrated area of collapsed gel spread stepwise between the anode and the wet zone. Marked irregularities and distortions of the protein zones occurred mostly along the central part of the plate. Satisfactory separations could be achieved at the sides of the gel layer.

In spite of the promising results (Fig. 1), it can be concluded that electroendosmosis still remains a substantial obstacle to routine electrofocusing in agarose gel, even when using purified agarose exhibiting low electroendosmosis. A higher degree of purification of agarose will be necessary for further progress in this direction. This conclusion is in accordance with a recent study[3] that was published during our experimental work. However, no detailed data concerning the technique are yet available.

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