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Note

First experience with the use of the Pharmacia PhastSystem for the characterization of haemoglobins by isoelectric focusing

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This paper reports our recent results achieved with the new PhastSystem (Pharmacia, Uppsala, Sweden)[1] during the characterization of purified stroma-free haemolysates of human erythrocytes, their lyophilizates after prolonged storage and their pyridoxalated and polycondensed derivatives by isoelectric focusing (IEF). We have performed similar experiments earlier on other instruments with very good analytical separations of haemoglobin subfractions[2–4]. Nevertheless, the PhastSystem promised further miniaturization of sample volumes and of pattern size and shortening of the experimental time, together with standardization and automation of the method. Our results supported these optimistic predictions.

EXPERIMENTAL

Lyophilized native human haemoglobin and its derivatives modified with pyridoxal-5-phosphate, glutaraldehyde and serum albumin were prepared as before[4]. The samples were desalted by gel filtration on Sephadex G-25 with distilled water as eluent. Analytical isoelectric focusing on PhastGel IEF 3–9 was performed in the PhastSystem using the procedure recommended by the manufacturer[1]. The following conditions were employed: Pre-focusing at 2000 V, 2.5 mA, 3.5 W (limiting) for 75 V h; 1 μl of sample per lane applied automatically at 200 V for 15 V h; final focusing at 2000 V, 2.5 mA, 3.5 W for 410 V h. The gel was stained automatically with PhastGel Blue R (Coomassie R-350) in the Development Unit of the PhastSystem according to the recommendations given in the operating manual and dried on Gelbond film. The focused bands were compared with the positions of proteins from an IEF calibration kit serving as reference for the determination of p*I* values.

RESULTS AND DISCUSSION

Fig. 1 shows a sharp separation and good reproducibility of the IEF fractionation of our samples. Native human oxyhaemoglobin was separated into its typical subfractions, which remained unchanged even after 3 years of storage in the freeze-

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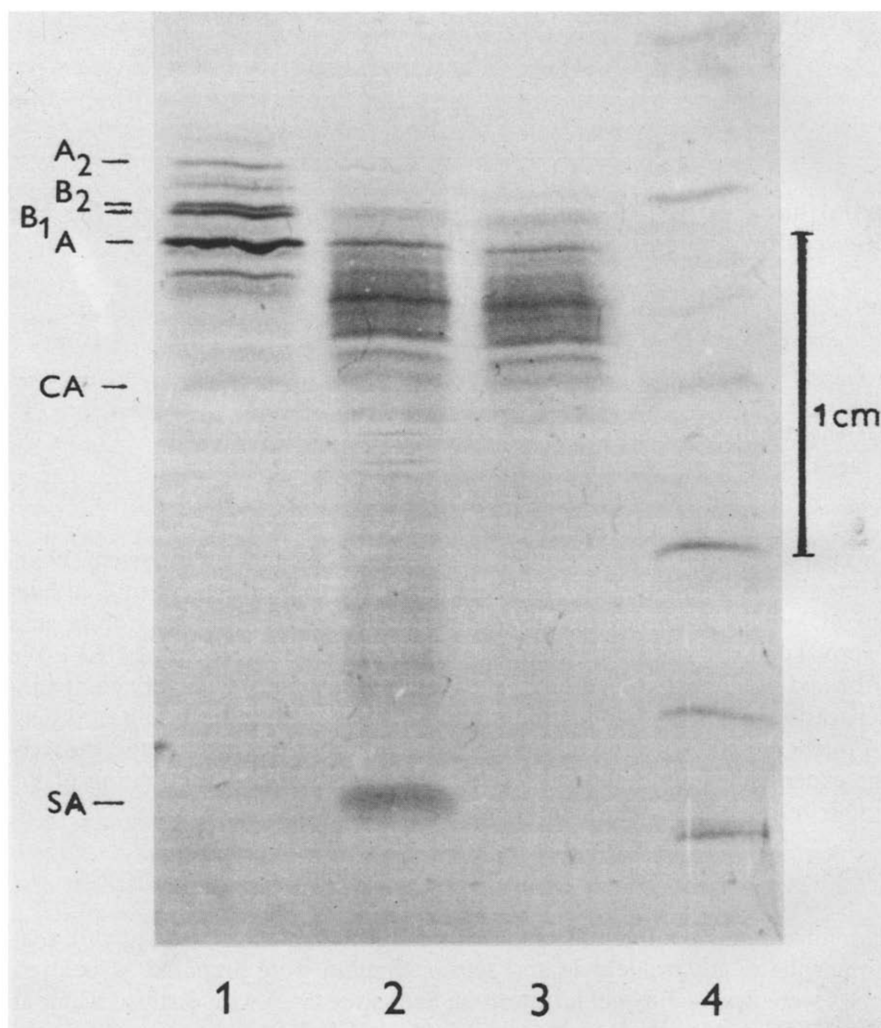

Fig. 1. IEF patterns of haemoglobin samples obtained using the PhastSystem. 1 = Native human oxyhaemoglobin lyophilized with sucrose, after 3 years of storage; 2 = pyridoxalated polycondensed mixture of human haemoglobin and serum albumin; 3 = pyridoxalated polycondensed human haemoglobin; 4 = calibration kit. PhastGel IEF 3–9, run for 30 min, staining with Coomassie R-350, 30 min. Typical haemoglobin subfractions are indicated by A and B, the position of carbonic anhydrase by CA and that of serum albumin by SA.

dried state with sucrose at 4°C[2]. Similarly, the pyridoxalated and polycondensed haemoglobin samples, with or without the addition of serum albumin, showed characteristic complex patterns corresponding to different chemically altered haemoglobin molecules[5]. Excellent separations were achieved in a substantially shorter time (1 h instead of about 5 h) and with less effort than before.

Our "oxyhaemoglobin kit" lyophilized with sucrose again proved most useful as a coloured p*I* marker for the region around pH 7, as shown in previous experi-

ments[2]. Its practicability for both scientific and educational purposes is connected with the visualization of the separation procedure during the run and the possibility of stopping the procedure at the most suitable moment. The sharpness of the separation of the two haemoglobin bands B_1 and B_2 in particular serves here as a criterion of the quality of a given IEF run.

In conclusion, we consider the PhastSystem to be an important step forward in the modernization, automation and miniaturization of the IEF microanalysis of complex protein mixtures.

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