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Gel isoelectric focusing of glycoprotein hormones*

Isoelectric focusing in polyacrylamide gels is a new analytical technique of high resolving power that has been applied to various proteins and complex protein mixtures[1–4]. Gel isoelectric focusing has proved to be a sensitive measure of the purity of proteins as well as a suitable method for the determination of their isoelectric points.

This communication deals with the isoelectric focusing of crude and highly purified human gonadotropins in a slab of large-pore polyacrylamide gel, essentially according to the method of AWDEH *et al.*[2], with a new selective staining procedure for proteins in the presence of carrier ampholytes and a new method of sample application in different pH regions.

Experimental

A slab of large-pore gel is used as a stabilizing medium. The Ampholine (LKB-Produkter, Stockholm) carrier ampholytes, covering the pH range 3–10, have a concentration of 2% (w/w).

Solution I consists of 100 g of acrylamide p.a. (Serva, Heidelberg), 2.7 g of N,N'-methylenebisacrylamide p.a. (Serva, Heidelberg) and distilled water to 300 ml. Solution II consists of 4 mg of riboflavin and distilled water to 100 ml.

Six ml of solution I, 2 ml of solution II and 2 ml of Ampholine (40%), pH 3–10, are added to 30 ml of distilled water. The gel mixture is transferred by pipette between two glass plates which are kept a distance of 1 mm apart by a polyethylene tube placed between them. In contrast to AWDEH *et al.*[2], we found that siliconizing one of the glass plates is unnecessary.

Photopolymerisation is allowed to proceed for 1.5 h under a fluorescent bulb positioned 15 cm away. One glass plate is then carefully removed.

Protein samples of 50–800 μg, dissolved in 20 μl of distilled water, are soaked in 1-cm² filter-paper discs which are put on the gel. As many as six samples may thus be run simultaneously. It is important to apply proteins of acidic pH to the part of the gel to become alkaline, and *vice versa*.

Isoelectric focusing is carried out at a constant potential of 330 V for 5–6 h at 4° in a moist plastics box. The current decreases from 32 mA to 2–3 mA during the run. The pH gradient is subsequently determined by punching a line of about 25 discs from the gel with a cork borer, and suspending them in 0.5 ml of twice-distilled water; the pH is measured with a micro glass electrode (405-M 3, Ingold, Frankfurt).

The gel slab is then immediately stained in a 0.1% solution of Coomassie Brilliant Blue R 250 (Serva, Heidelberg) in water: ethanol:acetic acid (50:45:5) for 20–30 min, preferably with mechanical agitation. The gel is loosened from the glass plate within the staining fixation solution. The slab is then washed thoroughly in the de-staining solution, water–ethanol–acetic acid (55:40:5) with continuous shaking. The solution is changed once. The background becomes clear within 2–3 h. The gel slab can be stored at 4° for several weeks.

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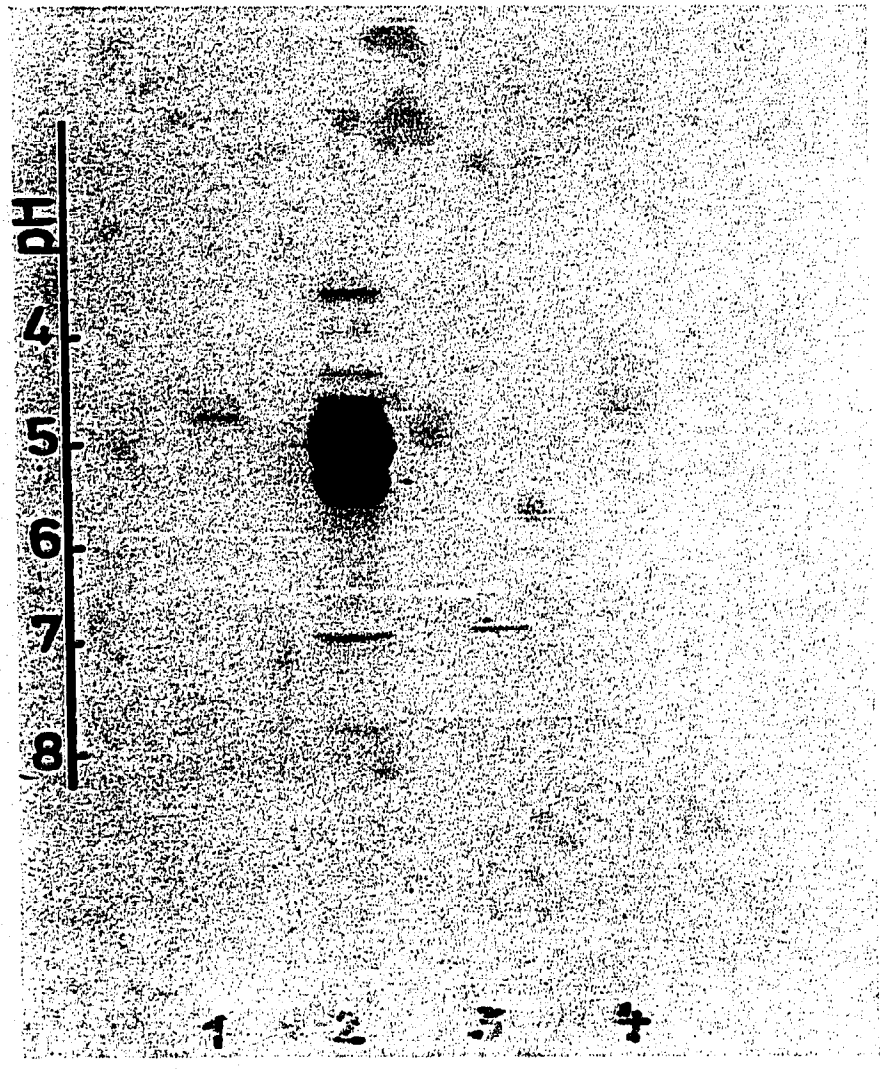

Fig. 1. Isoelectric focusing pattern of (1) human FSH, (2) crude gonadotropin (E_3), (3) human LH, (4) crude HCG in polyacrylamide gel, 2% Ampholine carrier ampholytes, pH range 3–10, staining with Coomassie Brillant Blue.

Results and discussion

Fig. 1 demonstrates the isoelectric focusing of gonadotropins in polyacrylamide gel. The following proteohormones were studied:

(1) Crude gonadotropin (E_3), extracted from human pituitaries, with a biological activity of 120 I.U./mg follicle stimulating hormone (FSH) and 430 I.U./mg luteinizing hormone (LH)[5].

(2) Highly purified luteinizing hormone, isolated[6] from E_3 by column isoelectric focusing in a sucrose density gradient in the pH range 3–10, containing 4200 I.U./mg LH by the OAAD-test[7] and less than 2 I.U./mg FSH.

(3) Highly purified FSH with a biological activity of 1200 I.U./mg, isolated[6] from E_3 by gel filtration on Sephadex G 100 followed by preparative column isoelectric focusing in the pH range 3–10 and a second run in the pH range 3–6.

(4) Crude human chorionic gonadotropin (HCG) with a biological activity of 3200 I.U./mg (Organon, Oss, The Netherlands).

The isoelectric focusing pattern of crude gonadotropin (E_3) shows a heterogeneous spectrum with at least 20 bands (Fig. 1, position 2). On disc electrophoresis (7.5%, pH 8.9), only 10 bands could be seen[8]. The components had isoelectric points in the pH range 3.6–8.4. Major contaminants of normal serum proteins could be identified as albumin, prealbumin and acid α_1-glycoprotein at pH 4.9, 4.7 and 3.6, respectively. The highly active LH showed a single strong band at a pH of 6.9 with only a faint trace of contaminants (Fig. 1, position 3).

The potent FSH revealed two distinct bands covering the very limited pH range 4.5–4.7 (Fig. 1, position 1). Crude HCG gave a complex pattern (Fig. 1, position 4). In agreement with the acidic nature of this glycoprotein hormone, owing to the large amount of sialic acid present[9], banding occurred in the narrow pH range 3.8–5.1. Although LH and HCG have the same biological effects, they show different isoelectric points.

A number of publications have dealt with the reproducibility of the separation of protein mixtures on gel isoelectric focusing, but only one author considered the aspect of sample placing at different pH values[10]. LEWIN found it advantageous to place pH-sensitive proteins as near as possible to their isoelectric points. In contrast to this, we observed denaturation and artefacts when proteins with pH 4.5 and below were placed near the anode, and cathodic reduction for proteins with alkaline pH when they were placed on the slab region at the cathodic end.

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