Journal of Chromatography, 311 (1984) 93—99
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 2235

DETECTION OF CHANGES IN RABBIT SERUM PROTEINS AFTER PARTIAL HEPATECTOMY BY MEANS OF TWO-DIMENSIONAL ELECTROPHORESIS UNDER NON-DENATURING CONDITIONS

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(Received March 16th, 1984)

SUMMARY

The changes in rabbit serum proteins after partial hepatectomy were examined by means of two-dimensional electrophoresis utilizing isoelectric focusing in a 4% polyacrylamide gel in the first dimension and a 4—30% pore gradient polyacrylamide gel in the second dimension. A rapid increase in seven proteins was observed after partial hepatectomy and a rapid decrease in two proteins. Major serum proteins, including albumin, immunoglobulin G, immunoglobulin M and α_2-macroglobulin, did not change.

The time course of the changes was examined using a densitometer; the maxima of the changes were observed on day 3 after partial hepatectomy.

INTRODUCTION

Since the first report of Higgins and Anderson [1] on the quantitative assessment of liver regeneration after partial hepatectomy, a large number of studies on the regenerating liver cells have been reported. However, what controls the proliferation after partial hepatectomy and how the rapid regenerative growth is stimulated are not yet fully clear. A series of specific substances is supposed to control the proliferation of liver cells, but it is also considered that metabolic changes after partial hepatectomy contribute to the stimulation of these substances. To answer this question, the changes in the serum concentration of various metabolites after partial hepatectomy have been studied [2—5]. The changes in serum proteins after partial hepatectomy, however, are not yet known except for some proteins [2—6] because of the insufficient resolution of the analytical techniques.

On the other hand, Manabe et al. [7] have described a two-dimensional

0378-4347/84/$03.00

electrophoretic technique without denaturing agent, and have shown that human plasma proteins can be resolved into about 250 spots. Since this technique does not use denaturing agents such as sodium dodecyl sulphate or urea during the electrophoretic run, it is suitable for the analysis of a mixture of soluble proteins without loss of their native physicochemical properties [8] or their biological activity [9].

In a previous paper [10] we applied this electrophoretic technique to analysing the changes in rat serum proteins after partial hepatectomy, and could detect changes in more than twenty serum proteins supposed to be closely related to the proliferation of liver cells. In this present paper we describe the changes in rabbit serum proteins after partial hepatectomy.

MATERIALS AND METHODS

Reagents

Ampholine (pH range 3.5—10) was obtained from LKB Produkter (Bromma, Sweden). Acrylamide, N,N′-methylenebisacrylamide, N,N,N′,N′-tetramethylethylenediamine (all special grade for electrophoresis), Tris base, glycine, sucrose and ammonium persulphate were purchased from Wako (Osaka, Japan). Coomassie Brilliant blue R-250 was purchased from Sigma (St. Louis, MO, U.S.A.).

Partial hepatectomy and serum samples

Male rabbits (Japanese White strain) weighing approximately 2 kg were used. Partial hepatectomy (about 60% of liver weight) was performed under light ether anaesthesia. After partial hepatectomy the rabbits were maintained for 1—20 days with food and water ad libitum. Sham operation was done in an identical manner to partial hepatectomy in terms of skin and muscle incision, manipulation of the liver and care of the wound. On days 0, 1, 2, 3, 4, 7, 10 and 20 after partial hepatectomy, blood (about 1 ml) was obtained from an ear vein using a disposable syringe. The blood was left to stand at 4°C for 30 min, and then centrifuged at 3000 *g* for 10 min. Sucrose was added to the serum to give a concentration of 10% (w/v), and the samples were stored at −20°C until use.

Two-dimensional electrophoresis

Two-dimensional electrophoresis was carried out as described in the previous report [10] with some modifications. Isoelectric focusing in the first dimension was performed on a gel column (14.5 cm × 0.5 cm I.D.). A 4% acrylamide (0.2% bisacrylamide) solution containing 2% Ampholine (pH range 3.5—10) and 0.05% ammonium persulphate was poured into the glass tube. After gelling, the bottom of the glass tube was covered with dialysis membrane, and placed in an electrophoresis chamber. Overlay solution (5% sucrose solution containing 2% Ampholine, 50 μl) was layered on top of the gel column and then the serum sample (50 μl) was applied below the overlay solution. The cathode electrode solution was 0.04 *M* sodium hydroxide, and the anode 0.01 *M* phosphoric acid. Electrophoresis was run at a 2-mA constant current per gel column for about 40 min (until the voltage reached 460 V), and then

460 V constant voltage for 20 h at 4°C. After electrophoresis, the gel was pushed out and placed directly on top of the second-dimension slab gel without equilibration.

Gradient polyacrylamide gel electrophoresis in the second dimension was performed with a slab gel apparatus which forms four slab gels of 12 cm long, 16 cm wide, 0.4 cm thick. A 4—30% acrylamide linear gradient (0.2% bisacrylamide) containing a 0—10% sucrose and a 0.05—0.025% ammonium persulphate linear gradient, was poured in about 50 min at 4°C. Gelling occurred in about 2 h in a water-bath at 30°C. The gradient gel buffer was 0.14 *M* Tris—HCl (pH 8.9), and the electrode buffer was 0.05 *M* Tris—0.38 *M* glycine (pH 8.3). Electrophoresis was run at a 36-mA constant current per slab gel for 20 h at 4°C.

Measurement of the pH gradient

The pH gradient was measured as follows. The first-dimension isoelectric focusing gel was duplicated for each sample. One gel was cut into 10-mm sections and these sections were placed in individual test tubes containing 2 ml of distilled water. These test tubes were allowed to stand for 2 h at room temperature, then the pH was measured on a pH meter.

Staining and destaining

Gels were stained for 24 h in 0.025% Coomassie Brilliant blue R-250—7% (v/v) acetic acid—50% (v/v) methanol. Destaining was done in 7% (v/v) acetic acid overnight at room temperature, and then at 80°C for 6 h. During the staining and the destaining the gel container was shaken gently.

Densitometry of Coomassie Brilliant blue stained spots

Densitometric quantitation of Coomassie Brilliant blue stained spots on the slab gel was carried out with a Shimadzu dual-wavelength thin-layer chromatographic scanner CS-900 (Shimadzu, Tokyo, Japan). Sample wavelength was 580 nm (reference wavelength 750 nm) and the Coomassie Brilliant blue stained spots were measured by transmission.

RESULTS

Several serum samples of normal rabbits (Japanese White strain, approximately 2 kg) were subjected to two-dimensional electrophoresis, the protein distributions were compared, and the positions of the serum proteins were reproducible. Fig. 1 shows an example of the two-dimensional electrophoretic pattern of normal rabbit serum. Major serum proteins were located on the gel by comparing the patterns with those of human plasma proteins [11]; some of these are shown in the figure.

The time course of the changes in the two-dimensional patterns of serum proteins after partial hepatectomy was examined. Serum samples obtained on days 1, 2, 3, 4, 7, 10 and 20 after partial hepatectomy were subjected to the two-dimensional electrophoresis and the protein distributions were compared with those of the normal serum pattern. Rapid changes were observed for several proteins; these are shown by arrows in Fig. 1 (proteins in area C are

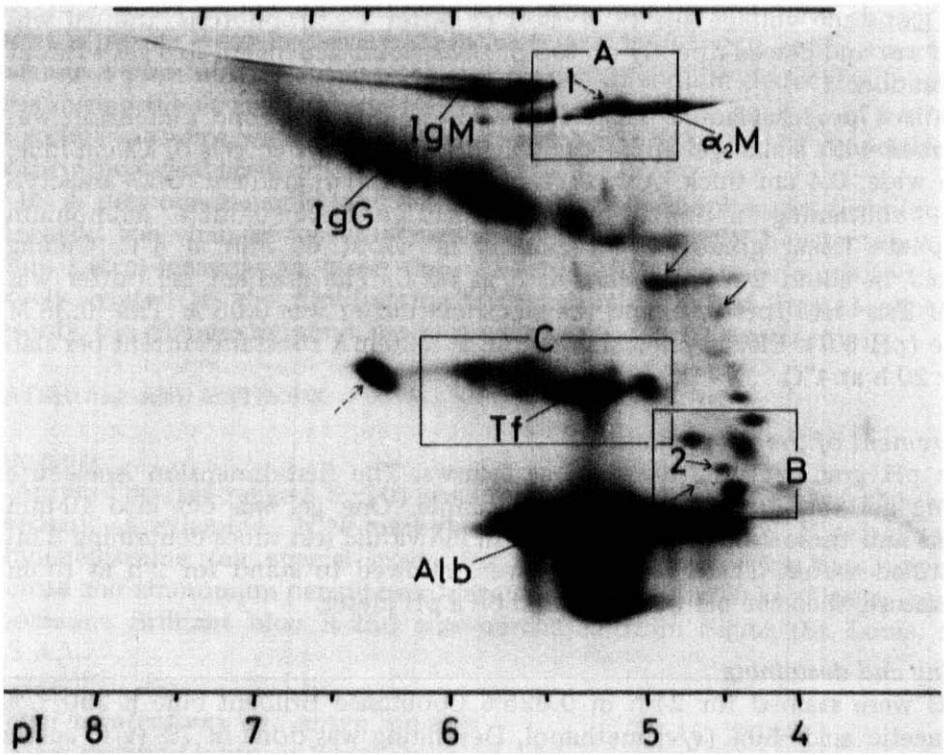

Fig. 1. Two-dimensional electrophoretic pattern of normal rabbit serum proteins. Dotted arrows indicate the decreased proteins and solid arrows indicate the increased proteins after partial hepatectomy. The positions of major serum proteins were tentatively located on the slab gel by comparing the two-dimensional electrophoretic pattern of rabbit serum with those of human plasma proteins [11]. IgM = immunoglobulin M, IgG = immunoglobulin G, α_2M = α_2-macroglobulin, Tf = transferrin, Alb = albumin.

shown in Fig. 2C). Dotted arrows show the proteins that are apparently decreased after partial hepatectomy and the solid arrows show the proteins that are apparently increased. Some major serum proteins, such as albumin, immunoglobulin G, immunoglobulin M and α_2-macroglobulin, did not change after partial hepatectomy.

Fig. 2 shows the time courses of the proteins particularly changed after partial hepatectomy. The proteins and the areas on the acrylamide slab gels are shown in Fig. 1. The protein of spot 1 rapidly decreased after partial hepatectomy, was minimal on day 3, then gradually increased and almost recovered its original level on day 20. The protein of spot 2 rapidly increased after partial hepatectomy, was maximal on day 3, and almost returned to its original level on day 20. Transferrin was observed as several spots over a wide pI range, but after partial hepatectomy distribution of its basic side changed more than that of its acidic side.

Quantitation of proteins on the acrylamide slab gels was carried out with a Shimadzu TLC scanner. Fig. 3 shows the time courses of the changes of protein spot 1 and protein spot 2. The extent of the changes was expressed as relative amount (amount before partial hepatectomy = 100%). It was demon-

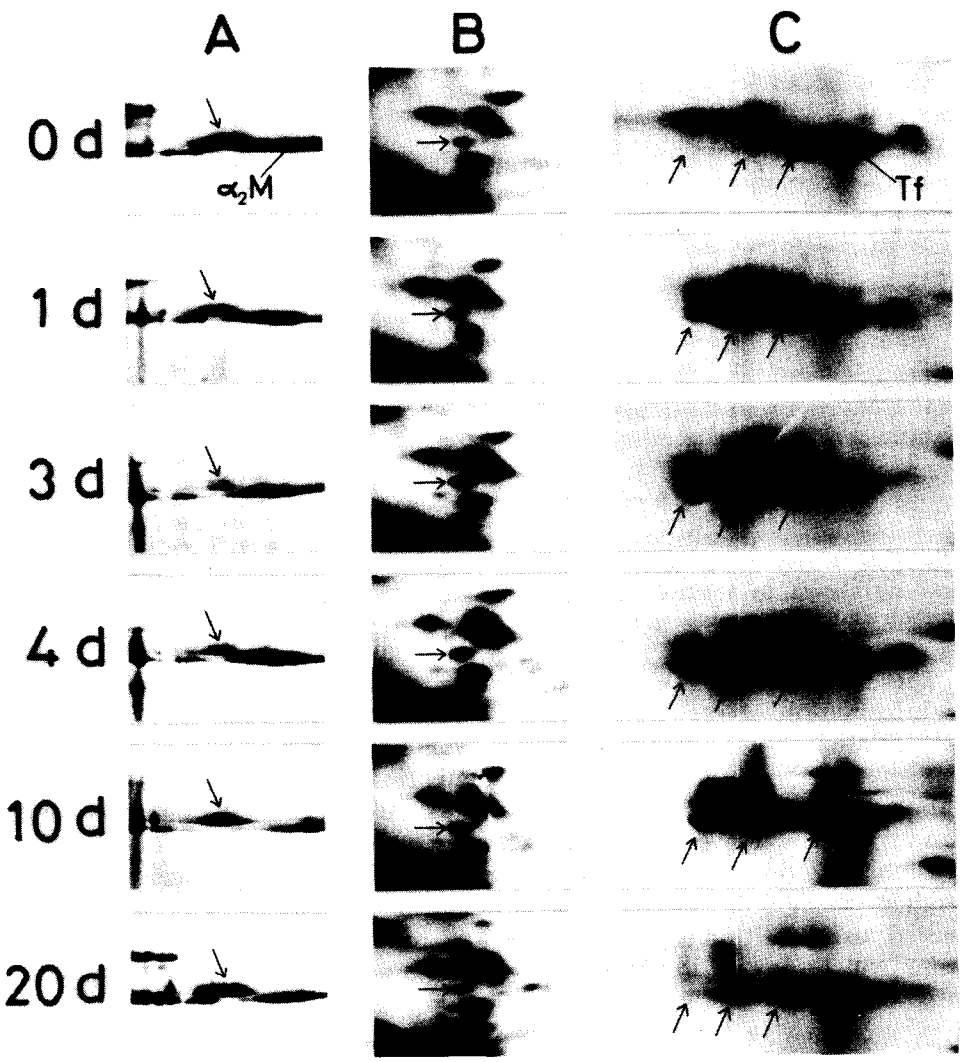

Fig. 2. Time courses of the changes in the two-dimensional pattern of rabbit serum proteins after partial hepatectomy. Serum samples taken on days 0, 1, 2, 3, 4, 7, 10 and 20 after partial hepatectomy were analysed by two-dimensional electrophoresis. Time-dependent changes on the gel sections in areas A, B and C (indicated in Fig. 1) are shown. Protein positions that changed rapidly after partial hepatectomy are indicated by arrows. α_2M = α_2-macroglobulin, Tf = transferrin.

strated that the protein amount of spot 1 decreased below 10% of its initial level on day 3, and the protein amount of spot 2 increased above 300% of its original level on day 3.

DISCUSSION

Changes in serum proteins after partial hepatectomy have been studied by means of one-dimensional electrophoretic techniques, such as cellulose acetate membrane electrophoresis [3] and polyacrylamide gel electrophoresis

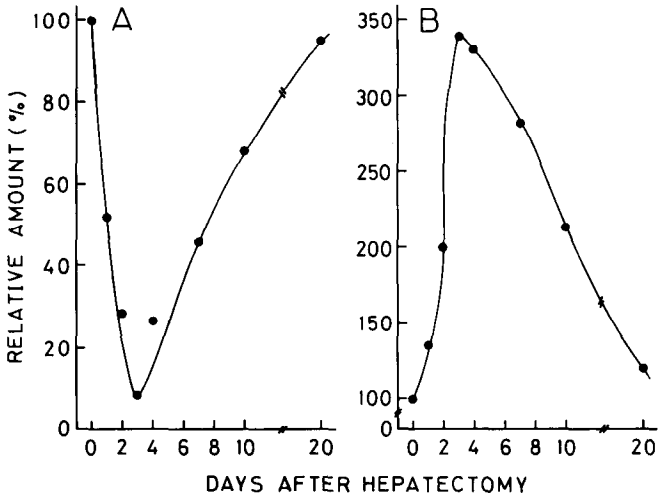

Fig. 3. Densitometric quantitation of proteins on acrylamide slab gels. The quantitation of protein spots 1 and 2 was carried out with a Shimadzu TLC scanner. Time-dependent changes are shown by relative amounts (amount before partial hepatectomy = 100%). (A) protein spot 1; (B) protein spot 2.

[5]. These one-dimensional electrophoretic techniques, however, could offer little information about the changes in the serum proteins supposed to be closely related to the proliferation of liver cells after partial hepatectomy. As shown in Figs. 1 and 2, the two-dimensional electrophoresis described in this paper can detect the changes in serum proteins after partial hepatectomy. Since this technique does not require equilibration of the first-dimension gel, there is no loss of proteins. Therefore, the accurate time-dependent changes in amounts of proteins on acrylamide slab gels can be measured using a thin-layer scanner. The quantitation of rapidly changed proteins will be useful in estimating the restoration of liver cells after partial hepatectomy.

It is also possible that the changes in serum proteins described above are due not only to partial hepatectomy but also to the operation wounds. Therefore, we analysed the serum of sham-operated rabbits by two-dimensional electrophoresis and compared the levels of serum proteins with those after partial hepatectomy. However, no changes were observed except for transferrin, which after sham operation showed changes similar to those shown in Fig. 2C, but the extent was not so much as that after partial hepatectomy. Although the function and role of these changes are still unclear, we suppose these proteins contribute, by themselves or with other substances, to the proliferation of liver cells after partial hepatectomy.

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