MODIFICATION OF RIBOSOMAL PROTEINS DURING LIVER REGENERATION

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SUMMARY

Analysis of the 2D gel electrophoretic pattern of ribosomal proteins from both the small and large subunit of rat liver were made at various times following partial hepatectomy. No changes were observed in the electrophoretic mobility of proteins from the 60S subunit during periods of 2 hr. to 72 hr. of liver regeneration. Changes were observed, however, in two proteins of the 40S subunit a short time after partial hepatectomy. Protein S_6 disappeared from its normal position and a new spot appeared as a more negative form as early as 2 hr. post regeneration. This modification persisted for at least 18 hr. At 72 hr., S_6 returned to its normal position. Protein S_2, on the other hand, underwent a different pattern of change during the early stages of liver regeneration. S_2 was observed to migrate as 2 spots at 2 hr. after partial hepatectomy and this pattern was preserved at the 4 hr. period. At 8 hr., the pattern was further modified to 2 spots which was distinct from the earlier change. This pattern was similar at 12 hr. At 18 hr. only the normal S_2 protein was observed. No further change in S_2 migration was observed at the 72 hr. period of liver regeneration.

INTRODUCTION

Following partial hepatectomy, a number of rapid morphological and biochemical changes occur in the remaining portion of the liver (1). These changes include an increased capacity of regenerating liver for protein synthesis (2,3), which appears to be directly related to the function of the ribosomal components of the protein synthetic system (2,3). Qualitative as well as quantitative changes have been observed in the proteins synthesized by regenerating liver (4,5).

The present studies were designed to determine whether modifications occur in the ribosomal protein components during liver regeneration. The two-dimensional (2-D) polyacrylamide gel electrophoresis technique developed by Kaltschmidt and Wittmann (6) affords an excellent opportunity to compare ribosomal proteins from different sources and make qualitative examinations of modification of these proteins.

MATERIALS AND METHODS

Female Sprague-Dawley rats (100-300 g) used in this study were maintained as previously described (7). Acrylamide was purchased from Eastman Kodak Company and doubly recrystallized from acetone before use. Urea solutions used in the electrophoresis procedure were deionized by passage through a LD-3 Demineralizer unit (Corning Glass Works).

Partial hepatectomy was performed while the animals were under light anesthesia (8). Control rats were sham-operated. Following surgery, the rats were allowed free access to food and water for 18 hr. at which time the livers were removed. The livers were chilled, weighed, minced in 2.5 volumes of cold Buffer II (250 mM sucrose, 100 mM KCl, 10 mM HEPES[I], 0.1 mM Disodium EDTA, 5 mM Mg $(CH_3CO_2)_2$, brought to pH 7.4 with KOH) and homogenized with a glass teflon homogenizer. The post mitochondrial supernatant fraction was prepared by centrifugation at 20,000 g for 10 min. The lipid layer was removed by aspiration and 0.1 volume of 15% DOC was added to the supernatant fraction and centrifuged at 20,000 g for 10 min. The lipid layer was again removed by aspiration and the supernatant fraction centrifuged at 150,000 g for 1.5 hr. to obtain the crude ribosomal pellet. This pellet was resuspended in PRM (20 mM HEPES, 100 mM KCl, 5 mM $Mg(CH_3CO_2)_2$, brought to pH 7.4 with KOH) to a concentration of approximately 200 A_{260} units/ml and dissociated into ribosomal subunits with puromycin and 500 mM KCl as described by Blobel and Sabatini (9).

I The abbreviations used are: HEPES, N-2-hydroxyethylpiperazine-N-2 ethanesulfonic acid; DOC, sodium deoxycholate.

This mixture was layered over a 10-30% linear sucrose gradient containing 300 mM KCl, 20 mM Tris-HCl pH 7.5, and 3 mM $Mg(CH_3CO_2)_2$ (80-100 A_{260} units/gradient) and centrifuged at 40,000 g for 16 hr. (Beckman SW27 rotor) at 25°C. The gradients were collected through an ISCO ultraviolet Monitor (Instrument Specialties Company) and the peaks corresponding to the 40S and 60S subunits pooled separately and pelleted at 100,000 g for 16 hr. (4°C). These subunits were used directly to prepare ribosomal proteins for electrophoresis or layered with a small volume of PRM and stored at -20°C.

Subunits (35 A_{260} units/ml) were suspended in PRM and 100 mM $MgCl_2$ and extracted with 66% acetic acid as described by Waller and Harris (10). Protein concentration was determined by the method of Groves et al (11). Electrophoresis was performed by a modification of the Kaltschmidt and Wittmann procedure (12). The separation gel (15 cm) was 5% acrylamide, and the spacer gel (1 cm) was 4.5%. Ribosomal proteins (750-950 ug) in 1-D Buffer (12) were diluted with an equal volume of 2% agarose and layered onto the spacer gel. The remaining space (to 18 cm) was filled with equal volume of 1-D buffer and 2% agarose. Gels were electrophoresed for 17.5 hr. at 3 mA/gel (room temperature). The cylindrical gels were equilibrated with 2-D buffer (12) and polymerized to a 16% acrylamide slab gel (18 cm x 0.2 cm) and electrophoresed at 140 volts for 43 hr. (room temperature). The gels were stained in 7% ethanol and 3% acetic acid containing 0.5% Aniline Blue Black. The gels were destained as described by Kaltschmidt and Wittmann (12).

RESULTS

Fingerprints of proteins from 40S subunits (Fig. 1) and 60S subunits (not shown) revealed 33 resolvable proteins from the small subunit and 40 from the large subunit. Since our gel procedure excludes the examination of acidic components, only basic proteins of each subunit will be considered in the present study.

The 60S liver ribosomal proteins obtained at various times following partial hepatectomy were compared with those obtained from sham-operated controls.

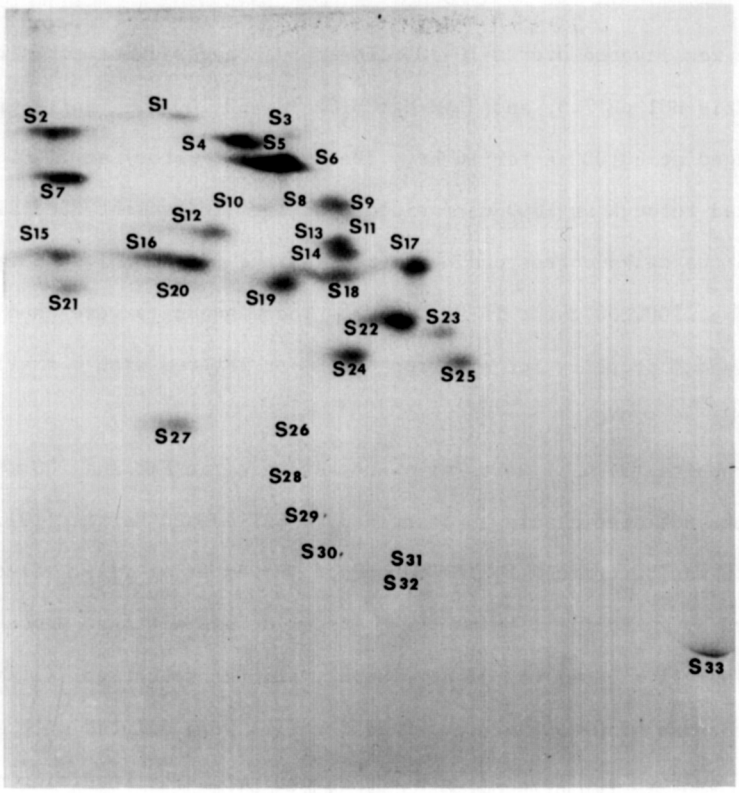

Fig. 1. Fingerprint of basic proteins from the 40S subunit of rat liver.

The electrophoretic patterns reveal that there were no significant changes of any of the 40 basic proteins throughout the 72 hr. period of liver regeneration. Several modifications were observed, however, when fingerprints of the 40S proteins were compared with controls following partial hepatectomy. As indicated in Fig. 2A-G, changes were noted in two proteins as early as 2 hr. following partial removal of the liver. These changes revealed a different pattern of modification of electrophoretic mobility over the period of liver regeneration for each of the two proteins. For the sake of clarity, only the upper portion of each gel pattern is presented since no changes were observed in any of the proteins that normally appear in the lower portion of the second dimension slab gel. At 2 hr. (Fig. 2B), the amount of normally migrating protein S_2 was decreased and a new protein spot appeared with a more positive charge to the right of the position of S_2 in the gel pattern. This pattern was duplicated for S_2 at 4 hr.

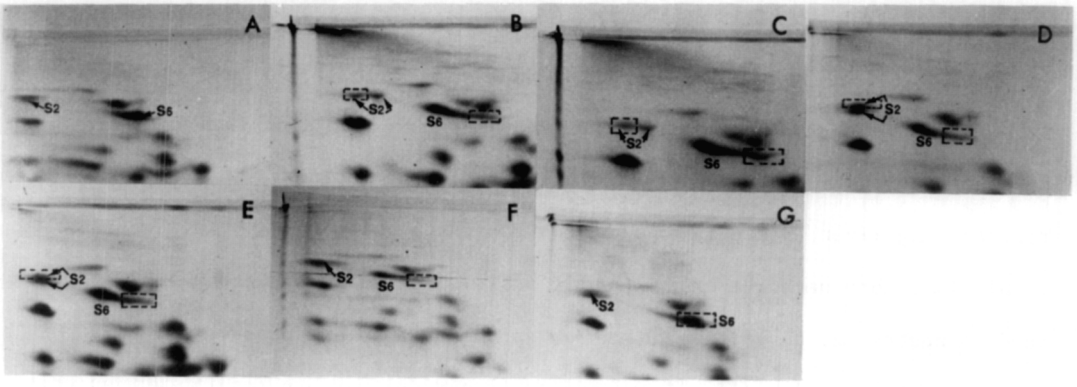

Fig. 2. Upper portion of fingerprint of proteins from 40S subunit at various times following partial hepatectomy. Arrows indicate the positions of the two proteins of interest. A. control 875 ug protein; B. 2 hr. 875 ug; C. 4 hr. 900 ug.; D. 8 hr. 875 ug.; E. 12 hr. 860 ug; F. 18 hr. 700 ug.; G. 72 hr. 850 ug.

post hepatectomy (Fig. 2C), however, at 8 hr. (Fig. 2D), the electrophoretic mobility pattern of the modified S_2 changed to another form which migrated in the gel at a position just below the normal S_2. This pattern remained constant at 12 hr. following partial hepatectomy (Fig. 2E), but at 18 hr. (Fig. 2F) protein S_2 appears to have reverted to its normal form (of only one spot which migrated to the same position as S_2 from sham-operated controls). No modification in the electrophoretic mobility of S_2 was noted at 72 hr. after partial hepatectomy (Fig. 2G).

Modification of electrophoretic mobility of protein S_6 of the 40S subunit during liver regeneration presented a simpler pattern of change. The modification of S_6 occurred as early as 2 hr. post hepatectomy (Fig. 2B), however, in contrast to the change in S_2, the modification of S_6 was to a form which had a more negative electrophoretic mobility than the normal S_6. This type of modification was maintained throughout the first 18 hr. of liver regeneration (Fig. 3B-F). By 72 hr. (Fig. 2G) the electrophoretic mobility of S_6 had reverted to the normally migrating form, although a small amount of streaking was observed in the gel, possibly an indication that the reversion was still not entirely complete.

DISCUSSION

A number of post translational modifications of proteins have been reported. Of these modifications, both phosphorylation (13-16) and acetylation (17) of ribosomal proteins from various sources have been described. Either phosphorylation or acetylation could account for the modification of S_6 in regenerating liver, since insertion of a phosphate group into protein would result in a more negative charge, while addition of an acetyl group would tend to neutralize a positive charge on an amino group and produce a less positively charged protein. A comparison of our gel pattern for the 40S subunit with that of Welfle et al (18) reveals that protein S_6 in our numbering system appears to be identical to S_9 in their system, and it is interesting to note that Stahl et al (15) observed that S_9 (our S_6) could be extensively phosphorylated in vitro, whether as an isolated protein or as a component of the 40S subunit or 80S monomer.

In the case of protein S_2, the modification appears to be more complex than that of S_6. S_2 appears to undergo a pattern of cyclic change during the first 18 hr. of liver regeneration. The first change observed at 2 hr. results in the disappearance of some of the normally migrating S_2 (Fig. 3B) and the appearance of another component which migrates as a protein more positively charged than normal S_2. Although we cannot state categorically that the second spot is a modification product of S_2, our gel procedure results in the quantitative migration of protein from the first dimension gel into the second dimension slab gel. Thus, the decrease in staining intensity of the normal S_2 and appearance of a new spot when equal amounts of protein are applied to the control and experimental gels (Fig. 3A and B) strongly suggest that the new spot is indeed a modified S_2. Since the new component migrates more rapidly toward the cathode in the first dimension than normal S_2, the initial modification of S_2 could be either a dephosphorylation or deacetylation.

Between 4 and 8 hr. post hepatectomy, a second modification occurs to the previously modified S_2, since this spot disappears from the gel pattern (Fig. 3D)

and a new spot appears directly below the normal position of S_2.

This migration pattern appears similar to that of L_7 and L_{12} for E. coli, described by Wittmann's group (19,20) for two proteins which are identical except for the presence of an acetyl group in the amino terminal residue of L_7 (21). This second modification of S_2 may be a deacetylation of the earlier changed S_2. Further work is required, however, to establish the chemical basis for these changes. As with the previous modification of S_2, this second modification is also short lived, since between 12 and 18 hr. post regeneration (Fig. 3E and F) this second modified form of S_2 also disappears and only the normal S_2 remains.

A recent paper by Gressner and Wool (22) has demonstrated that the modification of S_6 following partial hepatectomy is the result of phosphorylation. Their observations were made 22-24 hrs. after partial hepatectomy. Our recent studies indicate that the observed modification in S_6 occurs as early as 2 hrs. following partial removal of the liver. Our studies also indicate a modification in protein S_2. This change occurred again as early as 2 hrs. following partial hepatectomy. The migration pattern of S_2 had returned to normal by 18 hrs., consequently Wool's results do not document this alteration.

The physiological significance of these changes is not yet clear. It is interesting to speculate that these changes in the 40S subunit proteins may permit the ribosome to recognize specific types of mRNA and preferentially synthesize particular proteins. Several groups have obtained results suggesting that early during liver regeneration the rate of protein synthesis (23,24) increases dramatically, Scornik (25) presented evidence - with regenerating mouse liver - to show that although there is a larger proportion of ribosomes present as polyribosomes compared to normal liver, the in vivo rate of translation of polyribosomes of normal and regenerating liver is the same. Hill et al (26) has noted that the synthesis of albumin, the major export protein of the liver, is reduced 18-20 hours after partial hepatectomy relative to other proteins.

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