Two-Dimensional Gel Electrophoresis of Acid-extractable Nuclear Proteins of Regenerating and Thioacetamide-treated Rat Liver, Morris 9618A Hepatoma, and Walker 256 Carcinosarcoma

Lynn C. Yeoman, Charles W. Taylor, and Harris Busch

Department of Pharmacology, Baylor College of Medicine, Houston, Texas 77025

SUMMARY

The acid-soluble nuclear proteins of regenerating and thioacetamide-treated rat livers as well as the Morris 9618A hepatoma and the Walker 256 carcinosarcoma were extracted from citric acid-isolated nuclei with 0.4 N H₂SO₄. The nuclear extracts were analyzed by two-dimensional polyacrylamide gel electrophoresis. Although most of the protein spots were common to the livers and tumors studied, all of the rodent tumors were similar in their marked density of Spots C16-C18. In normal, regenerating, and thioacetamide-treated liver, the spots in this region were much less dense.

INTRODUCTION

The development of 2-dimensional electrophoresis methods for separation of nuclear and nucleolar proteins (2, 8, 11, 12) has provided a means for improved comparison of these proteins in tumors and other tissues (11). As might have been anticipated from their similar functions, the nucleolar proteins of normal liver and Novikoff hepatoma were remarkably similar in both distribution and relative amounts as indicated by spot density (8). However, studies on the whole nuclear acid extracts revealed a number of differences between the Novikoff hepatoma and the normal liver with respect to both density and distribution of the protein spots (11). As a further attempt to analyze these proteins, efforts are being made to improve both the technology for separating these proteins and determination of their composition. However, before proceeding toward characterization of any of the proteins of the Novikoff hepatoma, it seemed desirable to determine which of the differences from the normal liver might be related to growth and which, if any, might be related to the neoplastic process.

In the present study, the 2-dimensional gel electrophoretic system was used to analyze the nuclear proteins of the regenerating liver as an example of a normal growing tissue, the thioacetamide-treated liver as an example of a tissue with nucleolar hypertrophy, and accelerated nucleolar biosynthesis as well as 2 additional tumors, the highly differentiated, slowly growing Morris hepatoma 9618A and the rapidly growing Walker 256 carcinosarcoma.

The polyacrylamide gel patterns for these tissues provide evidence for many similarities in the proteins soluble in 0.4 N H₂SO₄. In particular, as might be suspected from its morphology, the patterns for the Morris hepatoma 9618A and normal and regenerating liver are very similar. However, it was of interest that, in the C region of each of the tumors studied, there was a dense area that was not found in any of the nontumor tissues.

MATERIALS AND METHODS

Preparation of Tumors and Other Tissues. Thioacetamide-treated rat livers were obtained from male, albino, 200-g rats from the Holtzman Company, Madison, Wis. Each rat was treated each day (i.p.) for 9 days with a dose of 50 mg of thioacetamide (Fisher Scientific Co., Fair Lawn, N. J.) per kg of rat body weight (1). Regenerating rat livers were obtained after partial hepatectomy was performed according to the procedures of Higgins and Anderson (4) and Muramatsu et al. (7). Approximately 70% of the liver from a male, albino, 200-g rat was removed and the remaining 30% was allowed to regenerate for 17 hr. The Walker 256 carcinosarcoma was implanted s.c. in 4 sites 6 days prior to the experiments and removed by dissection. The Morris hepatomas (9618A) were generously provided by Dr. Harold P. Morris. Normal rat livers were perfused and passed through a tissue press as previously described (11).

Isolation of 0.025 M Citric Acid Nuclei. The 0.5% citric acid method (10), in which a Tekmar SD-45K Super Dispa system is used for cell disruption, permits rapid isolation of nuclei that retain most of their nuclear proteins and yet are free of cytoplasmic contaminants as well as the outer layer of the nuclear envelope (10). The nuclei obtained by this method were of quality equivalent to those obtained by the 5% citric acid method (3). This procedure removes 23, 15, and 3% of the protein, RNA, and DNA, respectively, from rat liver nuclei prepared by the sucrose method as compared to 42, 19, and 14% protein, RNA, and
DNA, respectively, released by 5% citric acid from the same nuclei (10). Normal, thioacetamide-treated, and 17-hr regenerating rat livers were perfused with ice-cold 0.13 NaCl:0.005 KCl:0.008 MgCl₂ solution (6, 11). The perfused rat liver tissues were chopped and passed through a tissue press. After the Walker carcinosarcoma and Morris hepatomas were dissected, they were also passed through a tissue press. Novikoff hepatoma ascites fluid was collected by abdominal incision, and the cells were washed with NaCl:KCl:MgCl₂ solution buffer as previously described (11). All tissues were diluted 1:10 with 0.025 citric acid (pH 2.5) (5, 9, 10) and homogenized at 4° using a Tekmar SD-45K Super Dispax System (Tissumizer) for cell disruption with the G-456 generator (10). The time required for homogenization was 3 to 5 min, depending on the tissue. The nuclei obtained after homogenizing were further purified by centrifugation through sucrose (10).

**Extraction of 0.4 H₂SO₄-soluble Proteins.** Nuclei were immediately extracted with 10 volumes of 0.4 H₂SO₄ using a Teflon-glass homogenizer (11). The sulfuric acid extracts were dialyzed against 0.01 HCl and deionized water and lyophilized (11). The composition of the 0.4 sulfuric acid extracts was 91 to 98% protein, 2 to 9% RNA, and less than 1% DNA in each of the various systems studied.

**Two-Dimensional Polyacrylamide Gel Electrophoresis.** Two-dimensional polyacrylamide gels were prepared and run by the method of Orrick et al. (8). The gels were stained with 0.2% Coomassie brilliant blue R in acetic acid:methanol:water (1:5:5).

**RESULTS**

**Two-Dimensional Polyacrylamide Gel Analysis of 0.4 H₂SO₄-soluble Proteins of Normal Rat Liver Nuclei.** The 2-dimensional polyacrylamide gel pattern and composite drawing for the normal liver nuclei 0.4 H₂SO₄ extract are shown in Chart 1. As was pointed out previously (11), the pattern is divided into the A, B, and C regions with a total of 98 protein spots. The numbers designate spots seen in 0.4 H₂SO₄ extracts of normal liver and Novikoff hepatoma nucleoli (8), while lower case letters represent spots seen in whole nuclear acid extracts (11).

In this pattern, Spots GAR, Al, and A4 correspond in migration to the GAR (f₂a₁), AL (f₂a₂) and AR (f₃) histones (11). Marker spots in this pattern useful for orientation in the following patterns are A24, B13, and C23-24 in addition to GAR, Al, and A4.

**Two-Dimensional Polyacrylamide Gel Analysis of 0.4 H₂SO₄-soluble Proteins of Regenerating Rat Liver Nuclei.** The sulfuric acid-soluble nuclear proteins from the regenerating rat livers were separated by electrophoresis on 2-dimensional polyacrylamide gels. The pattern obtained shown in Chart 2A, as well as the composite drawing prepared from multiple samples and gels (Chart 2B), is very similar to that of normal liver (10). In the combined A, B, and C regions, a total of 88 spots was counted. Six dense spots, ARα, ARβ, ARc, ARd, ARe, and ARf, were found in the A region of the regenerating liver pattern that were not found in normal liver (11). In addition to the dense spots shown in Chart 2, a number of less dense spots were found in regenerating liver as compared to control normal liver (Chart 1) including 6 spots in the A region, 3 spots in the B region, and 3 spots in the C region.

**Two-Dimensional Polyacrylamide Gel Analysis of 0.4 H₂SO₄-soluble Proteins from Liver Nuclei of Thioacetamide-treated Rats.** The sulfuric acid-extractable nuclear proteins from the livers of rats that were given injections of thioacetamide daily for 9 days were analyzed on 2-dimensional gels. The pattern obtained is shown in Chart 3A, and the composite prepared from multiple samples and gels is shown in Chart 3B. A total of 91 spots was counted on this gel. Interestingly, no major spots were found in the thioacetamide-treated rat liver nuclear pattern that were different from those of the normal liver (Chart 1B).
Two-Dimensional Polyacrylamide Gel Analysis of 0.4 N H$_2$SO$_4$-soluble Proteins from Walker 256 Carcinosarcoma Tumor Nuclei. Analysis of the 0.4 N H$_2$SO$_4$-soluble nuclear proteins of Walker 256 carcinosarcoma tumors by 2-dimensional polyacrylamide gel electrophoresis showed there were 90 spots in this pattern (Chart 6). The dense region in the area of Spots C16, C17, and C18 was also present in the Walker 256 carcinosarcoma pattern (Chart 6A) but the key liver spots, B2, B5L, and Bp, were absent from this pattern.

Comparison of Nuclear Acid Extract Patterns from Liver and Tumor Nuclei. Several protein spots were found to be consistently different qualitatively. Spots B2, B5L, and Bp were found in acid extracts from normal rat liver (11), 17-hr regenerating rat liver, and livers of thioacetamide-treated rats; they were absent from the acid extracts of the Walker and Novikoff tumors, but the Morris hepatoma pattern contained Spots Bp and B5L. Spot B2 was not found in the Morris hepatoma.

Spots Aa and Ab, which coalesced at greater densities, were most intense in the thioacetamide-treated and regenerating liver nuclear patterns as well as the Walker 256 car-
DISCUSSION

The nuclear proteins extracted with 0.4 N H₂SO₄ from normal, regenerating, and thioacetamide-treated rat liver were compared with those from the Novikoff hepatoma, the Morris 9618A hepatoma, and the Walker 256 carcinosarcoma in this study by 2-dimensional polyacrylamide gel electrophoresis. Examination for the differences reported in a comparison of sulfuric acid-soluble proteins from normal rat liver and Novikoff hepatoma (11) revealed that, in each of the 3 liver tissues studied, Spots B2, B5L, and Bp were present. These spots were absent or present in trace amounts in the tumor lines examined, with the exception of B5L which was very intense in the Morris 9618A hepatoma. Of the hepatomas, the Morris hepatoma 9618A is remarkably phenotypically preserved with respect to cell and nuclear morphology compared to normal liver; it contained both the B5L and Bp spots but it lacked the B2 spot. It seems possible that the proteins in spots Bp and B5L may be involved in maintenance of the liver phenotype.

It is of special interest that in the 17-hr regenerating liver several new spots were reproducibly found in the A region. It remains to be determined whether these are modified histones, modified proteins of preribosomal particles, or other proteins. With those exceptions, the regenerating liver pattern was very similar to that of normal liver.

The high density of spots C16, C17, and C18, which were confluent in the gel patterns from the 3 rodent tumors studied (11), differentiated these patterns from the nontumorous tissues. These dense spots may represent increased amounts of a few components in the C regions of the tumors, i.e., a quantitative increase in the normal proteins in...
ACKNOWLEDGMENTS

The authors would like to thank Rose K. Busch for the transplantation of tumors.

REFERENCES

Two-Dimensional Gel Electrophoresis of Acid-extractable Nuclear Proteins of Regenerating and Thioacetamide-treated Rat Liver, Morris 9618A Hepatoma, and Walker 256 Carcinosarcoma

Lynn C. Yeoman, Charles W. Taylor and Harris Busch


Updated version Access the most recent version of this article at:
_http://cancerres.aacrjournals.org/content/34/2/424_