Chromatographic Patterns of Protein and Enzymes in Extracts of Rhabdomyosarcoma and Muscle in Mice

P. U. ANGELETTI, V. SUNTZEFF, AND B. W. MOORE

(Wernse Cancer Research Laboratory, Washington University Medical School, St. Louis, Mo.)

SUMMARY

Soluble proteins of rhabdomyosarcoma and normal muscle were chromatographed and separated into a number of peaks by gradient elution from DEAE cellulose. The chromatographic patterns were different in the two tissues studied.

Various enzymatic activities were determined in the fractions and localized in the chromatograms of both muscle and rhabdomyosarcoma.

Some of the enzymes tested appeared in multiple peaks and showed a pattern of distribution in tumor tissue different from that in the muscle.

The question frequently has been raised whether the protein and enzyme patterns of tumors and of comparable normal tissues differ from each other. Data obtained by various methods indicate that the amount and properties of the proteins may be altered in neoplastic tissues (21-23). An extensive investigation by Miller and co-workers on muscle and rhabdomyosarcoma extracts suggested that the protein composition of the neoplastic tissue differed from that of the normal (12, 13). This work provided a good foundation for further investigation with the method of column chromatography, to separate and identify the various protein components in extracts of these two tissues. Chromatography on anion exchange cellulose has been used in this laboratory to fractionate extracts of various normal and neoplastic tissues (1, 14, 15) and has been shown to be a useful method for the study of protein and enzyme patterns of different tissues. The work reported herein is concerned with the chromatographic patterns of proteins and several enzyme activities in extracts of muscle and of rhabdomyosarcoma in mice.

MATERIALS AND METHODS

Preparation of extract.—The muscle tumor used in this work had been first produced by injecting 20-methylcholanthrene in lard intramuscularly into mice of C3H strain, and has been transplanted for many generations. It was found to grow in 100 per cent of inoculated animals. Figures 1 and 2 show a section of the tumor as it appeared when the experiments were carried out; histological characteristics of this rhabdomyosarcoma did not show any visible change during successive transplantation; many fusiform strip-shaped cells, identified as neoplastic myoblast, and a great number of multinucleate giant cells were present.

For comparative purposes normal muscle was obtained from mice of the same strain and age, by dissecting muscles of fore and hind limbs. The tissues, especially the tumors, were carefully collected to avoid as much as possible any contamination from either necrotic or connective tissue.

In each experiment the material was immediately homogenized with three parts of 0.005 M Tris phosphate buffer, pH 8.0, in a “Virtis 45” homogenizer at half speed at 0° C. The homogenate was then centrifuged at 20,000 × g for 45 minutes and the clear supernatant removed (about 5 ml.) and dialyzed overnight with stirring against 1 liter of the same buffer.

Since myosin is not soluble below 0.02 M salt concentration, it was not extracted but was removed in the precipitate after centrifugation. This nonextractable material, when dissolved in 0.5 M NaCl, yielded an opalescent viscous solution, both from muscle as well as from rhabdomyosarcoma preparations.

Column chromatography.—The cellulose anion exchange columns were prepared as described by
Peterson and Sober (17, 20). The substituted cellulose, DEAE, as supplied, was washed with 1 M NaOH to remove colored material and fines and was further washed with distilled water to neutrality and dried with ethanol and ether. The capacity of the dried material was 1.02 meq/gm by titration. The prepared DEAE cellulose was stirred in water, adjusted to pH 8 and equilibrated with the Tris phosphate buffer. Columns (0.9 X 18 cm.) were prepared by packing a 2 per cent suspension at 10 pounds per square inch pressure. The dialyzed muscle and rhabdomyosarcoma extracts were applied to the columns in volumes of 3-5 ml. and allowed to soak in by gravity.

glucuronidase, glutamic oxaloacetic transaminase (GO-T), glutamic pyruvic transaminase (GP-T), lactic dehydrogenase (LDH), malic dehydrogenase (MDH), isocitric dehydrogenase (ICDH), and acid and alkaline phosphatases. The conditions employed for the assay of each enzyme and the references are given in Table 1. The apparent recovery of the protein was about 80 per cent, and that of the enzyme activities ranged from 70 to 100 per cent.

RESULTS

Protein and nucleic acid distribution.—Four muscle extracts and four extracts of rhabdomyosarcoma were fractionated. Chart 1 shows a typical chromatogram of rhabdomyosarcoma extract. The first protein peak, followed by some other smaller components, represents the part of the protein not strongly bound to the column and eluted by the starting buffer. With the salt gradient the remaining proteins were separated into a number of peaks, each one appearing at similar chloride concentrations in all the experiments. Nucleic acid, showing a typical absorbance with 280/260 ratios of about 0.5, appeared at a chloride concentration of about 0.5 M. Hemoglobin was spread out in a zone between 0.05 and 0.08 M NaCl.

Chromatography of muscle soluble proteins gave a significantly different pattern. As shown in Chart 2, a large portion of the muscle protein was weakly bound to the column, was eluted by the starting buffer, and appeared in two major peaks.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>mM Substrate</th>
<th>Buffer</th>
<th>pH</th>
<th>Measured</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose 6-phosphate dehydrogenase</td>
<td>Glucose-6-phosphate</td>
<td>5</td>
<td>2-amino-3-methyl-1-3-propanediol</td>
<td>9.5</td>
<td>TPNH</td>
<td>(5)</td>
</tr>
<tr>
<td>6-Phosphogluconate dehydrogenase</td>
<td>6-Phosphogluconate</td>
<td>1</td>
<td>Tris</td>
<td>8.2</td>
<td>TPNH</td>
<td>(10)</td>
</tr>
<tr>
<td>Isocitric dehydrogenase</td>
<td>D-Isocitrate</td>
<td>0.75</td>
<td>Tris</td>
<td>8.1</td>
<td>TPNH</td>
<td>(16)</td>
</tr>
<tr>
<td>Glutamic pyruvic transaminase</td>
<td>L-Alanine+</td>
<td>20</td>
<td>Phosphate</td>
<td>7.4</td>
<td>DPNH</td>
<td>(96)</td>
</tr>
<tr>
<td>Glutamic oxaloacetic transaminase</td>
<td>L-Aspartate+</td>
<td>20</td>
<td>Phosphate</td>
<td>7.4</td>
<td>DPNH</td>
<td>(9)</td>
</tr>
<tr>
<td>Beta-glucuronidase</td>
<td>Phenolphthalein-beta-glucuronide</td>
<td>1</td>
<td>Acetate</td>
<td>5.0</td>
<td>Phenolphthalein</td>
<td>(7)</td>
</tr>
<tr>
<td>Malic dehydrogenase</td>
<td>L-malate</td>
<td>200</td>
<td>Pyrophosphate</td>
<td>9.3</td>
<td>DPNH</td>
<td>(6)</td>
</tr>
<tr>
<td>Lactic dehydrogenase</td>
<td>Pyruvate</td>
<td>1</td>
<td>Tris</td>
<td>7.5</td>
<td>DPNH</td>
<td>(9)</td>
</tr>
<tr>
<td>a-Glycerol-phosphate dehydrogenase</td>
<td>Dihydroxyacetone phosphate</td>
<td>0.3</td>
<td>Triethanolamine</td>
<td>7.5</td>
<td>DPNH</td>
<td>(9)</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>p-Nitrophenylphosphate</td>
<td>10</td>
<td>Glycine</td>
<td>10.5</td>
<td>p-nitrophenol</td>
<td>(3)</td>
</tr>
<tr>
<td>Acid phosphatase</td>
<td>p-Nitrophenylphosphate</td>
<td>10</td>
<td>Citrate</td>
<td>4.8</td>
<td>p-nitrophenol</td>
<td>(3)</td>
</tr>
</tbody>
</table>

The columns were eluted with about 70 ml of the starting buffer; then a salt gradient, with slowly increasing slope, up to a final concentration of 1 M NaCl, 0.05 M sodium phosphate, pH 6.5 was used to elute the remaining material. The gradient was produced as reported elsewhere (14). The flow rate was approximately 10 ml/hour, and fractions of about 3-ml volume were collected with a fraction collector.

Chemical assays in the various fractions.—Protein concentration was determined in each fraction by the method of Lowry et al. (11). Optical densities were determined at 260 and 280 mµ to localize the nucleic acid. Heme proteins were determined by absorbancy measurement at 415 mµ. Several enzyme activities were assayed in each fraction: glucose-6-phosphate dehydrogenase (G-6-PDH), 6-phosphogluconic dehydrogenase (6-PGDH), β-glucuronidase, glutamic oxaloacetic transaminase (GO-T), glutamic pyruvic transaminase (GP-T), lactic dehydrogenase (LDH), malic dehydrogenase (MDH), isocitric dehydrogenase (ICDH), and acid and alkaline phosphatases. The conditions employed for the assay of each enzyme and the references are given in Table 1. The apparent recovery of the protein was about 80 per cent, and that of the enzyme activities ranged from 70 to 100 per cent.

RESULTS

Protein and nucleic acid distribution.—Four muscle extracts and four extracts of rhabdomyosarcoma were fractionated. Chart 1 shows a typical chromatogram of rhabdomyosarcoma extract. The first protein peak, followed by some other smaller components, represents the part of the protein not strongly bound to the column and eluted by the starting buffer. With the salt gradient the remaining proteins were separated into a number of peaks, each one appearing at similar chloride concentrations in all the experiments. Nucleic acid, showing a typical absorbance with 280/260 ratios of about 0.5, appeared at a chloride concentration of about 0.5 M. Hemoglobin was spread out in a zone between 0.05 and 0.08 M NaCl.

Chromatography of muscle soluble proteins gave a significantly different pattern. As shown in Chart 2, a large portion of the muscle protein was weakly bound to the column, was eluted by the starting buffer, and appeared in two major...
peaks followed by some smaller components. With the salt gradient, the remaining proteins appeared as several peaks and were mostly eluted before a concentration of 0.18 NaCl. Nucleic acids were localized at about 0.55 M NaCl. Material with absorbance at 413 μm appeared in the first protein peak and later in a zone between 0.05 and 0.08 M NaCl. The distribution of the muscle proteins as compared with the rhabdomyosarcoma is also shown in Table 2 as relative amounts of the proteins in various regions of DEAE chromatograms.

**Distribution of enzyme activities.**—Some of the enzymes assayed were localized in a few fractions and appeared as one sharp peak. Some others were separated into multiple distinct peaks, eluted at different chloride concentrations (Charts 1 and 2). LDH activity was localized with the first protein peak in a few closely spaced peaks in both muscle and rhabdomyosarcoma chromatograms. β-Glucuronidase appeared as one sharp peak at about 0.08 M Cl⁻ in all chromatograms. The activity, however, was at least 5 times higher in

---

**Chart 1.**—Rhabdomyosarcoma chromatogram; protein and enzyme activity distribution; 80 mg. of protein were applied to the column. Enzyme activities are expressed in μmoles/ml/hr.
tumor preparations. Isoctric dehydrogenase and glutamic pyruvic transaminase appeared in both tumor and muscle chromatograms in a wide region between 0.1 and 0.16 M Cl\(^-\) for the transaminase and between 0.06 and 0.08 M Cl\(^-\) for the dehydrogenase.

The enzyme, 6-phosphogluconic dehydrogenase, was localized in one sharp peak at 0.04 M Cl\(^-\) in the chromatograms of rhabdomyosarcoma. In the muscle preparation a very low activity of the same enzyme was detected at the same point.

Glutamic oxaloacetic transaminase activity separated into two zones. The first one in the basic protein portion appeared as only one peak in the tumor chromatogram, but as two close but distinct peaks in normal muscle. A second zone of activity was localized in all the chromatograms soon after the beginning of the gradient (Charts 1 and 2).

Malic dehydrogenase activity separated in both muscle and tumor chromatograms into two regions with no consistent differences in the relative peak heights (Charts 1 and 2). Acid phosphatase ac-
tivity also showed multiple forms, appearing in muscle chromatograms both in the basic protein fraction and as another smaller peak at about 0.09 M Cl⁻. In the tumor chromatograms the activity was almost equally divided between the first peak and several closely spaced peaks between 0.09 and 0.17 M Cl⁻. Alkaline phosphatase was detected in measurable amounts only in the rhabdomyosarcoma extract and was localized as shown in Chart 1.

α-Glycerol-phosphate dehydrogenase was present in muscle extracts and was spread out in a zone between 0.01 and 0.07 M NaCl. This enzyme was not found in measurable amounts in the tumor extract.

Glucose-6-phosphate dehydrogenase activity was found significantly increased in the rhabdomyosarcoma preparations, and in the chromatogram it separated into three distinct peaks at 0.1, 0.2, and 0.26 M NaCl. In the muscle chromatograms the glucose-6-phosphate dehydrogenase activity was localized in only one peak at 0.1 M NaCl (Charts 1 and 2), with no trace of activity in the 0.2 and 0.26 M Cl⁻ regions.

**DISCUSSION**

These data give further evidence that the protein composition of the rhabdomyosarcoma differs from that of the homologous tissue from which it originally derived. Under the conditions employed a large part of the muscle protein was not bound to the DEAE-cellulose. This fraction, which represents proteins having zero or positive charge, may include myogen and myoglobin. It was also found that some of the glycolytic enzymes were present in this fraction. In the rhabdomyosarcoma pattern the relative amount of this basic component of the soluble protein was reduced and represented only 15–20 per cent of the total recovered protein. This difference may be accounted for by the different degree of differentiation of the two tissues, since some highly specialized proteins of normal adult muscle may be missing in the tumor.

In the muscle chromatograms most of the proteins appeared between 0 and 0.18 M NaCl, but in the rhabdomyosarcoma chromatograms much of the protein was eluted at higher chloride concentration and was separated into a greater number of components. It is interesting to note that by means of electrophoretic analysis the soluble proteins extracted from rhabdomyosarcoma were separated into a larger number of components than those of the normal muscle (Miller et al.) (13).

A comparison of the rhabdomyosarcoma chromatograms with those of different types of squamous-cell carcinomas of the skin demonstrates a great similarity in their protein distribution (1). Some of the enzymes assayed showed, besides quantitative differences in their activities, a different localization in the chromatograms, e.g., glucose-6-phosphate dehydrogenase. The hexosemorphosphatase shunt in neoplastic tissue is known to have a role as a metabolic cycle alternative to the citric acid cycle for the aerobic metabolism of glucose-6-phosphate as well as a pathway for producing the pentose phosphate required in the formation of nucleic acids (18, 19). In the neoplastic liver, Weber and Cantero found the activity of this enzyme increased fivefold compared with that of the normal liver (24, 25). In our experiments the glucose-6-phosphate dehydrogenase activity was also significantly increased in the rhabdomyosarcoma extracts and was separated into three distinct peaks. In the muscle chromatograms, all the activity was localized in only one peak, corresponding to the first one of the tumor. Whether these separate peaks correspond to different forms of the same enzyme, distinct from one another, or to the same enzyme bound to other molecules, is a matter being investigated in this laboratory. It has been shown that the differences between the several forms separated from liver are not interconvertible if they are rechromatographed and therefore must not be loosely bound complexes (15). The increase of glucose-6-phosphate dehydrogenase in the tumor can be accounted for by the appearance of the last two peaks which are absent in normal muscle.

Alpha-glycerol phosphate dehydrogenase activity, well localized in the muscle chromatograms, was not detected in the various fractions of rhab-
domyosarcoma. This finding is in agreement with previous investigations (4) which reported a very low level of this enzyme in other malignant tissues.

On the contrary, alkaline phosphatase was detected in the tumor chromatograms in several distinct peaks but was not found in measurable amounts in the normal muscle.

In conclusion, within the limits of our investigation, the over-all pattern of the proteins as well as of the enzyme activities assayed appeared to be significantly different in the rhabdomyosarcoma than in the muscle tissue. The significance of these enzymes appearing in several chromatographically separable forms is not known at present.

The data herein presented suggest that such a difference may be of qualitative nature.

REFERENCES


13. ———. Electrophoretic and Viscosity Measurements. Ibid., pp. 44-54.


19. ———. Alternative Pathways of Glucose Metabolism. II. Nucleotides from the Acid-Soluble Fraction of Normal and Tumor Tissue and Studies on Nucleic Acid Synthesis in Tumors. Ibid., pp. 66-74.


Chromatographic Patterns of Protein and Enzymes in Extracts of Rhabdomyosarcoma and Muscle in Mice

P. U. Angeletti, V. Suntzeff and B. W. Moore


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/20/8_Part_1/1229.citation

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.