Chromatographic Profiles of Deoxyribonucleic Acid Preparations from Rat and Mouse Tissues

SAUL KIT*

From the Section of Nucleoprotein Metabolism, Department of Biochemistry, The University of Texas M. D. Anderson Hospital and Tumor Institute, Houston, Texas

(Received for publication, November 18, 1959)

Experiments by Bendich et al. (1, 2) and by Polli and Shooter (3, 4) have suggested that the deoxyribonucleic acids might be tissue specific. Bendich et al. (1) found that the DNA of rat spleen, kidney, brain, and intestine could be distinguished by their chromatographic profiles on diethylaminoethyl-cellulose. The chromatographic differences between rat kidney and brain DNA were particularly pronounced. Polli and Shooter (3) studied the sedimentation coefficient distribution curves at infinite dilution of DNA preparations from normal human leucocytes or spleen, and from human lymphatic leukemia or myeloid leukemia. Differences between the samples were observed and the following hypotheses were suggested to explain the differences: (a) Variations were associated with the average degree of maturity of the cells; (b) variations were due to the presence of different proportions of the types of cells, or (c) specific changes occurred in the deoxyribonucleic acid of all the cells.

It is apparent that problems of considerable genetic importance are raised by the above results. However, the differences in the DNA chromatographic profiles of various rat tissues have not been confirmed by Kondo and Osawa (5). The fractionation of DNA on substituted cellulose anion exchangers has also been under study in this laboratory (6-11). Several Ecteola-cellulose* anion exchangers of varying nitrogen content and exchange capacity have been employed to study the DNA chromatographic profiles of normal tissues and tumors. DNA preparations from the following rodent tissues have been investigated: rat spleen, kidney, lung, liver, and thymus. In agreement with the results of Kondo and Osawa (5), it was observed that the DNA chromatographic profiles of the various tissues of the same animal were rather similar.

EXPERIMENTAL

Preparation of DNA—DNA was prepared from the tissues of adult rats and mice. The animals had been fed ad libitum on standard laboratory diets before being killed. The method of Kirby (12) with minor modifications (6) was used to prepare the DNA. Approximately 20 mg of DNA were freshly prepared for each chromatographic run. Tissues were excised from 2 to 4 rats or from 20 to 30 normal mice as rapidly as possible and frozen in a Dry-Ice-ethyl Cellosolve bath (ethylene glycol monoethyl ether). The tissues were homogenized at 4-5°C with a loose fitting Potter-Elvehjem all glass homogenizer in 12 volumes of 6% sodium p-aminosalicylate. To the homogenate, an equal volume of 90% phenol was added and the suspension was stirred at 22°C for 1 hour. The suspension was centrifuged at 3000 r.p.m. in the HR-1 Refrigerated International Centrifuge and the aqueous layer was withdrawn. The phenol phase was reextracted with a small volume of 6% sodium p-aminosalicylate. In all subsequent purification steps, the DNA was dissolved in 0.01 M NaCl rather than in distilled water. The DNA was reprecipitated several times from a 4% sodium acetate solution and incubated at 4°C for 18 hours with crystalline ribonuclease (1 to 2 mg per g wet weight of the original tissue). The DNA was again precipitated from solution, redissolved, and separated from polysaccharides by extraction with 2-methoxyethanol (methyl Cellosolve) from potassium phosphate solution (12).

Over 90% of the DNA was extracted with sodium p-aminosalicylate and phenol and the final yield exceeded 70%. The DNA preparations were contaminated with about 5% RNA and 1% or less of protein. The protein content or the chromatographic properties of the samples were not changed, however, by treating the DNA with chymotrypsin (6). The ratios of purines to pyrimidines and of adenine plus cytosine to guanine plus thymine were approximately one (7). The E1°25 of the DNA preparations was 200 ± 13. Upon heating dilute solutions (about 0.005%) in 0.01 M or 0.05 M NaCl to a temperature of 90 to 100°C, an irreversible increase of about 35% in the optical absorption was observed (13). For the samples studied here, this transition was sharp, and took place between 85-98°C. The sedimentation constants of several DNA preparations were measured at concentrations of 0.03 to 0.1% DNA with the Spinco model B ultracentrifuge and Schlieren optics. The average sedimentation coefficient obtained by extrapolating the curve for 1/ε vs. against DNA concentration to zero DNA concentration, was 22 × 10^{-12} second. The intrinsic viscosities of the DNA preparations were determined with Cannon-Manning Calibrated Semimicro viscometers of 0.5 mi approximate charge, an efflux time for water at 25°C of approximately 300 seconds, and a shear gradient of about 600 sec^{-1}. The average intrinsic viscosity was 49 deciters per g (29°C, 0.2 M NaCl, 0.01 M phosphate, pH 7).

Chromatography—Details concerning the chromatographic

* Aided in part by grants from the American Cancer Society (P30A), the Leukemia Society, Incorporated, and the National Cancer Institute (C-4238). The author is indebted to Andrew Cox for expert technical assistance.

1 See “Experimental.”
procedures and a critical discussion of the methodology have been presented elsewhere (6). Two Ecteola-Solka Floe anion exchangers were employed: (a) Exchanger B-30; nitrogen content, 0.55%, exchange capacity, 0.39 meq per g; and (b) Exchanger B-39; nitrogen content, 0.42%, exchange capacity, 0.30 meq per g. The exchangers were purchased from Brown Company. Approximately 500 mg of exchanger were used for each column in the case of the B-39 exchanger, but 750 mg were employed in the case of the B-30 exchanger. The Ecteola-cellulose was thoroughly washed with water and suspended in 1 N NaOH before use. Excess alkali was removed by repeated washings with water and the exchangers were finally equilibrated with 0.05 M NaCl for 0.01 mg NaCl in 0.001 M phosphate buffer, pH 7.

Approximately 2.5 to 3.5 mg of freshly prepared samples of DNA (equivalent to 50,000 to 70,000 optical density units at 258 m\(\mu\) in the Beckman DK-2 spectrophotometer) were dissolved in the phosphate-buffered 0.05 M sodium chloride solution, and applied to the B-39 Ecteola-cellulose columns. Half this amount of DNA dissolved in the phosphate-buffered 0.05 M sodium chloride solution was added to the B-30 exchanger. Recovery of ultraviolet-absorbing material from the columns was in almost every instance greater than 90%.

The solvent flow through the columns was under the force of gravity. Five-milliliter aliquots were collected with the use of the DNA of mouse spleen and melanoma S91. As was observed in experiments with exchanger B-39, the elution profiles of the four tissues were small. For a check, one experiment was carried out on exchanger B-30, with the use of the DNA of mouse spleen and melanoma S91. The chromatographic profiles, obtained with Ecteola-cellulose, B-30, are shown in Fig. 4. From 15 to 40% of the DNA of rat tissues was eluted by eluents 3 and 4 (0.2 and 0.4 M NaCl, 0.001 M sodium phosphate), and from 55 to 60% by eluents 8 to 11 (2 M NaCl, 0.01 to 0.3 M NH\(_4\)). The differences in the elution profiles of the four tissues were small.

The DNA chromatographic profiles on exchanger, B-30, are shown in Fig. 4. From 15 to 21% of the DNA was eluted by eluents 3 and 4 (0.2 and 0.4 M NaCl, 0.001 M sodium phosphate), and from 55 to 60% by eluents 8 to 11 (2 M NaCl, 0.01 to 0.3 M NH\(_4\))). The differences in the elution profiles of the four tissues were small.

RESULTS AND DISCUSSION

Typical DNA chromatographic profiles, obtained with Ecteola-cellulose exchanger, B-39, are shown in Fig. 1. One peak is eluted by 0.6 M NaCl and 0.001 M phosphate, and four or more peaks by 2 M NaCl and NH\(_4\) solutions of increasing alkalinity. Approximately 60% of the DNA is eluted from this exchanger by 2.0 M NaCl and 0.1 to 0.4 M NH\(_4\) (eluent numbers 7 to 10 of Table II). The chromatographic profiles of DNA preparations from mouse thymus and lung were rather similar (Fig. 1).

In Figs. 2 to 4, the cumulative total of the DNA eluted from the exchangers is plotted against the eluent numbers which are shown in Tables I and II. The elution profiles of the DNA preparations shown in Fig. 2 were obtained with the stronger exchanger, B-39 (0.55% nitrogen; 0.39 meq per g exchange capacity) whereas those shown in Figs. 3 and 4 were obtained with exchanger, B-30 (0.42% nitrogen; exchange capacity, 0.30 meq per g). Fig. 2 shows that with exchanger B-39, about 15% of the DNA of rat tissues was eluted by the first three eluents (0.05 to 0.6 M NaCl) but about 60% by eluents 7 to 10 (2 M NaCl and 0.1 to 0.4 M NH\(_4\)). However, with the weaker exchanger, B-30, 27 to 33% of the DNA was eluted by the first 5 eluents (0.01 to 0.6 M NaCl) and about 55% by eluents 8 to 11 (2 M NaCl and 0.01 to 0.3 M NH\(_4\)) (Fig. 3). Yet, with either exchanger, the DNA chromatographic elution profiles of rat brain, kidney, and spleen were very similar.

Exu- Total

<table>
<thead>
<tr>
<th>Eluent No.</th>
<th>Volume</th>
<th>Composition</th>
<th>pH</th>
<th>Approximate flow rate per hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30</td>
<td>0.01 M NaCl, 0.001 M sodium phosphate</td>
<td>7.0</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>0.05 M NaCl, 0.001 M sodium phosphate</td>
<td>7.0</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>0.2 M NaCl, 0.001 M sodium phosphate</td>
<td>7.0</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>60</td>
<td>0.4 M NaCl, 0.001 M sodium phosphate</td>
<td>7.0</td>
<td>6.7</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>0.6 M NaCl, 0.001 M sodium phosphate</td>
<td>7.0</td>
<td>10</td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td>0.8 M NaCl, 0.001 M sodium phosphate</td>
<td>7.0</td>
<td>6.7</td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>2.0 M NaCl, 0.001 M sodium phosphate</td>
<td>7.0</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>30</td>
<td>0.01 M NH(_4), 2.0 M NaCl</td>
<td>10.0</td>
<td>5</td>
</tr>
<tr>
<td>9</td>
<td>60</td>
<td>0.1 M NH(_4), 2.0 M NaCl</td>
<td>10.0</td>
<td>6.7</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>0.2 M NH(_4), 2.0 M NaCl</td>
<td>10.0</td>
<td>10</td>
</tr>
<tr>
<td>11</td>
<td>30</td>
<td>0.3 M NH(_4), 2.0 M NaCl</td>
<td>10.0</td>
<td>10</td>
</tr>
<tr>
<td>12</td>
<td>30</td>
<td>0.4 M NH(_4), 2.0 M NaCl</td>
<td>10.0</td>
<td>10</td>
</tr>
<tr>
<td>13</td>
<td>30</td>
<td>1.0 M NH(_4), 2.0 M NaCl</td>
<td>11.1</td>
<td>5</td>
</tr>
<tr>
<td>14</td>
<td>60</td>
<td>0.5 M NaOH</td>
<td>6.7</td>
<td></td>
</tr>
</tbody>
</table>

The elution profiles of mouse liver, kidney, lung, and spleen on Ecteola-cellulose, B-30 are shown in Fig. 4. From 15 to 21% of the DNA was eluted by eluents 3 and 4 (0.2 and 0.4 M NaCl, 0.001 M phosphate), and from 55 to 60% by eluents 8 to 11 (2 M NaCl, 0.01 to 0.3 M NH\(_4\)). The differences in the elution profiles of the four tissues were small.

The DNA chromatographic profiles on exchanger, B-30, of diploid and tetraploid lymphomas, diploid and tetraploid carcinomas, melanomas S91 and S91A, and normal spleen cells have been described previously (7). The chromatographic profiles of all of the above tissues were rather similar. As a further check, one experiment was carried out on exchanger B-30, with the use of the DNA of mouse spleen and melanomas S91. As was observed in experiments with exchanger B-39, the elution profiles of mouse spleen and melanoma DNA on exchanger B-30 were almost identical.
Differences between rat kidney and brain DNA of the magnitude reported by Bendich et al. (1) were not confirmed by our experiments. In this respect, our results are in agreement with those of Kondo and Osawa (5). A number of factors may account for the discrepancies between the laboratories:

1. The methods used to prepare the DNA were not the same.

---

**Fig. 1.** DNA chromatographic profiles on Ecteola-cellulose (B-39) (Brown Company) of Akr mouse thymus and lung. Optical density was recorded at 258 nm. 62,000 optical density units of DNA were applied to each column. Recovery of optical density units from column: 74% and 77%, respectively (see Table II).

**Fig. 2.** DNA elution profiles of rat brain, spleen, and kidney on Ecteola-cellulose B-39 (0.39 meq per g exchange capacity, 0.55% nitrogen). Approximately 63,800, 63,000, and 62,800 optical density units were applied to each column. Recovery of optical density units from column: 90%, 90%, and 91%, respectively.

**Fig. 3.** DNA elution profiles of rat brain, spleen, and kidney on 750 mg of Ecteola-cellulose B-30 (0.30 meq per g exchange capacity, 0.42 nitrogen). The figures shown represent the averages of two determinations. Recoveries of optical density units from columns: 90%, 106%, and 104%, respectively (see Table I).

**Fig. 4.** DNA chromatographic elution profiles of mouse lung, kidney, liver, and spleen on 750 mg of Ecteola-cellulose B-30 (0.30 meq per g exchange capacity, 0.42 nitrogen). The results represent the averages of two determinations for each of the tissues except for mouse spleen. Average recoveries of optical density units from columns: 114%, 98%, 105%, and 111%, respectively.
The method of Kay et al. (14) or Mirsky and Pollister (15) were employed by Kondo and Osawa (5); the methods of Schwan-der and Signer (16) or of Kay et al. (14) by Bendich et al. (1), and the method of Kirby (1) by this laboratory. It cannot be assumed that DNA prepared from the same tissue by different methods or even that DNA prepared from different tissues by the same method will necessarily give products of equal purity and integrity (6, 17).

The Kirby phenol p-aminosalicylate method (12) of preparing DNA has the following advantages: (a) the extraction of DNA from tissues is essentially complete and the final yield is very high; (b) the possibility of partial degradation by nuclease is minimized (6). On the other hand, many of the common methods of preparing DNA involve conditions in which nuclease activity is uncontrolled or the methods are objectionable because of the use of alkali. The dissociation of DNA from lipoproteins by anionic detergents is slow without the action of these enzymes rather than on some catalytic power of the detergent to rupture the bonds between protein and DNA (18-20). In methods with alkali or heat, the alkali or the heating could bring about the cleavage. In milder methods involving prolonged extraction periods, it is very likely that mitochondrial enzyme action could play a role. Frick (21) has critically studied some commonly used methods of preparing DNA. Nucleoprotein was first prepared by extracting thymus tissue with 1 m NaCl and then precipitating with 0.14 m NaCl. The resulting nucleoprotein was then repeatedly deproteinized with a mixture of amyl alcohol and chloroform. If the work was carried out in the cold, a very low yield of DNA was obtained. In order to obtain a higher yield, it was necessary to resort to hydrolysis with 0.5% sodium carbonate at 50-55° for 1 to 2 hours before starting the chloroform treatment. If no hydrolysis was applied, the yield of DNA corresponded to the amount of protein in dissociation equilibrium with the nucleoprotein. In Hammarsten’s method, the nucleoprotein used as starting material was obtained by extracting the tissue three times with distilled water. The nucleoprotein was then precipitated from the extract with calcium chloride. The nucleoprotein was dissociated from the protein with saturated NaCl. Frick (21) concluded that although Hammarsten’s method gave a good yield, it also depended on enzymatic activity in the starting material.

2. The protein contamination of the samples varied. All of the methods of preparing DNA result in products contaminated by some protein. The DNA prepared in this laboratory by the Kirby method contained 1% or less of protein. Butler et al. (22) reported that three DNA samples prepared by the detergent method (dodecyl sulfate) contained 0.08, 0.2, and 0.8% protein. Protein contamination of DNA prepared according to Hammarsten may be relatively great (21). The presence of even small amounts of protein may markedly modify the physical chemical properties of DNA preparations because of cross linking. Butler et al. (22) observed that the distribution of sedimentation constants was decreased in three “dodecyl sulfate DNA preparations” which were treated with chymotrypsin. The addition of chymotrypsin to DNA which had been deproteinized by the Sevag procedure caused a definite change in the distribution curve of sedimentation coefficients, indicating that the properties of the latter product were still influenced by the presence of protein. Hermans (23) has observed reductions in the average molecular weight as determined by light scattering of various DNA preparations including one sample which was deproteinized with phenol. Significantly, however, the chromatographic elution profiles of the DNA preparations employed in the present studies were not modified by prior chymotrypsin treatment of the DNA (6).

3. The chromatographic procedures were different. A schedule of gradient elution was employed by Bendich et al. (1, 2), but discontinuous elution was employed by ourselves and by Kondo and Osawa (5).

4. The properties of the Ecteola-cellulose were not the same. Experiments were carried out in this laboratory with such exchangers of high and of low exchange capacity and nitrogen content. In addition, an experiment was performed with the exchanger, SF-4, which was obtained through the kindness of Dr. Aaron Bendich. The exchanger SF-4 manifested properties intermediate between that of exchanger B-30 and B-39. The DNA elution profiles were somewhat shifted towards the left when the exchangers of lower nitrogen content were employed but the chromatographic profiles of the various tissues were similar on any given exchanger.

The elution of ultraviolet-absorbing material from the Ecteola-cellulose columns exceeded 90% in almost every instance. It is worth emphasizing that alkaline eluents (pH 10 to 11) were required for the elution of most of the DNA. Since the DNA was exposed to these eluting solutions for hours, it seems possible that configurational changes, or other modifications in the structure of the DNA, may have taken place during chromatography. In some instances, the recovery of ultraviolet absorbing material from the columns was as high as 114%. A recovery in excess of 100% suggests that elution is accompanied by denaturation of DNA, which indeed would be anticipated from the use of alkaline eluents. The chromatographic profiles of heat denatured DNA differ markedly from native DNA (13). Denaturation of DNA during chromatography might also be manifested by an apparent increase in the heterogeneity of the samples. Clearly, there is an imperative need for anion exchangers from which DNA can be eluted by neutral sodium chloride solutions.

It is possible that intrinsic differences exist in the chromatographic properties of the DNA of different tissues of the same animal. Such differences were not detectable, however, in the DNA preparations studied in this laboratory. In view of the complexities involved in the preparation and chromatographic analyses of these macromolecules (6), extreme caution is indicated in extrapolating from the apparent differences which one may sometimes observe between particular DNA preparations and the properties of DNA in situ.

SUMMARY

Preparations of deoxyribonucleic acid were obtained by extracting rat brain, kidney, or spleen, and mouse lung, liver, spleen, kidney, or thymus with p-aminosalicylate and phenol. The preparations were chromatographed on Ecteola-cellulose anion exchangers of low and of high nitrogen content and exchange capacity. With either exchanger, the chromatographic elution profiles of the various tissues obtained from the same animal were very similar.
REFERENCES

Chromatographic Profiles of Deoxyribonucleic Acid Preparations from Rat and Mouse Tissues
Saul Kit