A study of the DNA of dogfish corneal epithelium

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Small Sephadex G-200 columns were employed to purify milligram quantities of DNA extracted from dogfish cornea epithelium with sodium dodecyl sulfate, and studies of the composition and physical properties were made. \( ^3 \text{H} \) thymidine and \( ^{125} \text{I} \) deoxyuridine were incorporated into epithelial DNA under simple in vitro circumstances. The incorporation of thymidine depended upon the temperature and oxygen tension of the incubation medium.

The epithelium of the dogfish cornea is ideally suited for studies on the chemistry and metabolism of DNA. As shown in Fig. 1, approximately 12 layers of cells whose nuclei are rich in dark Feulgen-staining material (DNA) make up nearly half the thickness of the cornea. Furthermore, both the content and rate of synthesis of DNA in this tissue are greater than those of mammalian and fish corneas.\(^{10}\) This report summarizes recent chemical studies on dogfish cornea epithelial DNA and on the rate of incorporation of precursors into DNA under in vitro conditions.

Methods and materials

Corneal epithelium was scraped from the eyes of freshly killed dogfish with a scalpel. When used for chemical studies, large groups of whole epithelia were pooled and immediately frozen. These epithelia were later homogenized in several volumes of distilled water with a Dounce glass homogenizer and the \( 600 \times g \) sediment was extracted at \( 60^\circ \text{C.} \) with 0.41 per cent sodium dodecyl sulfate in 0.9 per cent NaCl for one hour. After the denatured protein was precipitated by adjusting the NaCl concentration of the suspension to 1M and removed by centrifugation at \( 2,000 \times g \); one volume of cold ethanol was added to the supernatant to precipitate the DNA.\(^{1} \) The RNA which always coprecipitated along with the DNA was removed after hydrolysis in 0.3M KOH by the process of gel filtration described in Results.

DNA was determined by the microindole technique of Ceriotti\(^{2} \) and RNA was determined by the orcinol procedure.\(^{3} \) The Lowry\(^{4} \) technique was used to determine protein and ultraviolet absorption was measured with a Beckman DU spectrophotometer.

Sedimentation rates were determined with a Beckman-Spinco Model E analytical ultracentrifuge at \( 20^\circ \text{C.} \) and at a speed of 42,040 r.p.m. DNA base composition was obtained by the melting point determination described by Marmur and Doty,\(^{5} \) or by hydrolyzing the purified DNA with 70 per cent HClO\(_4 \) at \( 100^\circ \text{C.} \) for one hour\(^{6} \) and then separating the resultant bases by descending paper chromatography with the isopropanol:HCl:H\(_2\)O (65:35:1) system.

For in vitro incubations, groups of six to eight epithelia were combined and promptly dropped
DNA of dogfish corneal epithelium

Results

The average wet weight of an adult dogfish corneal epithelium was found to be 22.5 mg, as compared with 42.5 mg of stroma (25 determinations). One whole epithelium contained 59 μg of DNA (average of 20) for a concentration of 0.26 per cent of the wet weight or 1 per cent of the dry weight. The RNA:DNA ratio was found to be 2.0 as compared to ratios of 3 and 4 found for rabbit and calf corneal epithelia, respectively.

Fig. 2 represents the elution pattern obtained when an 0.25 ml. sample containing 200 μg of epithelial RNA and 400 μg of DNA (treated with 0.3M KOH for 18 hours at 37° C.) was filtered through a column of Sephadex G-200 (7 cm. × 0.5 cm.) with 0.025M tris buffer (pH 7.4) as the suspending and elution medium. Each fraction collected contained 0.16 ml. and the optical density at 260 m/μ was read for every third tube after a fivefold dilution with 0.01N HCl. With this procedure, 98 per cent of the DNA applied to the column was recovered in the first 4.0 ml. of eluent, while the remaining ribonucleotides came off in amounts.

Fig. 2. Elution pattern obtained by passing a mixture of dogfish cornea epithelial DNA plus KOH-hydrolyzed RNA through a column of Sephadex G-200 (see Results for details). DNA was eluted in the first 4 ml. of tris buffer; RNA in the next 7.5 ml.
Fig. 3. Schlieren pattern obtained with the Beckman-Spinco analytical ultracentrifuge for a solution of DNA in water (0.1 per cent) at 20° C. The print was made 16 minutes after rotation at 42,040 r.p.m.

Table I. Rates of incorporation of 3H thymidine and 125I deoxyuridine into dogfish corneal epithelium at 20° C. in elasmobranch Ringer’s solution over a 5 hour period

<table>
<thead>
<tr>
<th>Substance incorporated</th>
<th>Level in incubation medium</th>
<th>Incorporation rate (pmoles per mg. DNA per hr.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3H thymidine</td>
<td>10^-7 M</td>
<td>1.5 x 10^-7</td>
</tr>
<tr>
<td>125I deoxyuridine</td>
<td>10^-7 M</td>
<td>0</td>
</tr>
<tr>
<td>125I deoxyuridine</td>
<td>10^-5 M</td>
<td>2.4 x 10^-6</td>
</tr>
</tbody>
</table>

the next 7.5 ml. The DNA had a 258:280 absorption ratio of 2.0, a phosphorus content of 9.4 per cent, an $E_{260}$ value of 8,400 at 260 m$_4$, and a sedimentation rate of 46s (Fig. 3), giving a molecular weight of approximately two million. Base composition analysis gave a 43 mole per cent guanine (G) plus cytosine (C) and a 57 per cent adenine (A) plus thymine (T) content and a ratio of 6-amino (A + C) to 6-keto (G + T) group bases of 1.02. From melting point data, a GC content of 41 ± 1 mole per cent was obtained.

When groups of epithelia were incubated with 10^-7 M 3H thymidine, 1.5 x 10^-7 pmoles per milligram DNA per hour were incorporated during a 5 hour incubation time. 125I deoxyuridine was not incorporated into DNA at a level of 10^-6 M, but, when the concentration was increased tenfold, it was incorporated into the DNA at 2.4 x 10^-6 pmoles per milligram DNA per hour (Table I).

As shown in Table II, the incorporation of 3H thymidine into corneal epithelial DNA was dependent upon the oxygen tension and the temperature of the incubation medium. When N$_2$ replaced O$_2$ at 20° C, the rate of incorporation of thymidine was depressed by 45 per cent; when the incubation was carried out at 0° C under O$_2$, incorporation was inhibited by 55 per cent; and when N$_2$ was used at 0° C, a 78 per cent reduction of incorporation was achieved.

Discussion

This report describes basic techniques which will be useful in elucidating the effects of chemical and physical agents on the physical chemistry and metabolic activity of corneal epithelial DNA.

The multilayered nature of dogfish corneal epithelium and its relatively high DNA content have made it possible to obtain quantities of purified DNA suitable for the determination of its composition and approximate molecular weight. Purifi-

Table II. Effects of cooling and lowered oxygen tension on the incorporation rate of 3H thymidine (10^-7 M) into dogfish corneal epithelium in elasmobranch Ringer’s solution over a 5 hour period

<table>
<thead>
<tr>
<th>Conditions</th>
<th>O$_2$ (%)</th>
<th>N$_2$ (%)</th>
<th>CO$_2$ (%)</th>
<th>No. of experiments</th>
<th>Incorporation rate (c.p.m. per mg. DNA per hr.)</th>
<th>Per cent inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>20° C.</td>
<td>95</td>
<td>95</td>
<td>5</td>
<td>3</td>
<td>95</td>
<td>0</td>
</tr>
<tr>
<td>20° C.</td>
<td>95</td>
<td>95</td>
<td>5</td>
<td>3</td>
<td>50</td>
<td>45</td>
</tr>
<tr>
<td>0° C.</td>
<td>95</td>
<td>95</td>
<td>5</td>
<td>3</td>
<td>47</td>
<td>55</td>
</tr>
<tr>
<td>0° C.</td>
<td>95</td>
<td>95</td>
<td>5</td>
<td>2</td>
<td>13</td>
<td>78</td>
</tr>
</tbody>
</table>

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cation of DNA with Sephadex gel filtration allowed the removal of extraneous RNA without excessive dilution of sample. Dogfish DNA was found to be similar in composition to salmon and herring DNA.

In vitro incorporation of labeled DNA precursors such as $^3$H thymidine and $^{125}$I deoxynucleotide was dependent upon appropriate conditions of temperature and oxygen tension. Comparisons of rates of incorporation of base analogues with those of natural bases, estimations of the dose dependency of analogue incorporation, and studies on the effects of excessive concentrations of base analogues on the incorporation of naturally occurring bases can now be made. Several preliminary experiments indicated that when high concentrations of iododeoxyuridine were added to incubation medium containing $^3$H thymidine, the incorporation of thymidine into epithelial DNA was markedly depressed, probably as a result of competition between the two precursors for sites of incorporation into DNA molecules.

REFERENCES