AN UNUSUAL PROPERTY OF CHROMATIN ISOLATED FROM MAMMALIAN SALIVARY GLANDS.*

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Summary. Chromatin from mammalian salivary glands has been isolated and found to exhibit an absolute requirement for NaCl or KCl for its template activity. By chemical analysis this chromatin shows no difference in its basic protein complements as compared with liver chromatin. The absolute requirement of monovalent ions for the template activity of a chromatin has not been observed previously.

Introduction. Chromosomes, as they are isolated *in vitro*, are known as chromatin (1). This chromatin has been known for some time to carry out both DNA replication and RNA synthesis. It has been isolated from tissues such as pea plant (2), rat liver (3), calf thymus (4), chick embryo (5), and ascites tumor cells (6). These chromatins, when transcribed *in vitro* with RNA polymerase from *E. coli*, were shown to be capable of generating RNA of base sequences similar to that transcribed *in vivo* (7). The conditions for such a transcription were shown earlier (7).

In this communication the isolation of submaxillary salivary gland chromatin is being described. It exhibits a unique property of requiring monovalent ions (NaCl or KCl) for its template activity.

Methods. Submaxillary salivary glands were excised from 15-20 day old New Zealand white rabbits. Chromatin was then prepared from these glands by a modification of the method of Bekhor et al (7); where 0.25 M sucrose

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and 0.001 M $MgCl_2$ were maintained throughout the washing procedure. This chromatin was further purified through 1.7 M sucrose as described previously (7). The purified chromatin is partially soluble in 0.01 M Tris buffer, pH 8.0, and it is not solubilized any further upon shearing. Hence chromatin and not nucleohistone is utilized in these studies.

Liver chromatin from these rabbits was prepared by the method of Marushige and Bonner (3). RNA polymerase was purified from early log phase E. coli B by a modification of the method of Chamberlin and Berg (8). The new method will be published elsewhere. The basic change instituted in the procedure was that of lysing where dependency of RNA polymerase on exogenous DNA was increased by about tenfold over that obtained by the above method.

The assay mixture is as that described by Bekhor et al (7). Where necessary NaCl or KCl was added to the reaction mixture up to a concentration of 400 mM. Protein free DNA was prepared from the liver by the method of Marmur (9); disc gel electrophoresis was performed as described by Reisfield et al (10); protein assay was done by the method of Lowry et al (11); RNA by the orcinol procedure (12); and DNA by the method of Dische and Schwarz (13). The nucleotides were purchased from Schwarz BioResearch, Inc.; protamine sulfate and pronase free of nucleases from CalBioChem, Inc.; acrylamide and bisacrylamide from Eastman Organic Chemicals.

Results and Discussion. At the onset of this investigation, salivary gland chromatin (SGC) exhibited a template activity of zero in the reaction mixture described by Bekhor et al (7). Coincidently, SGC was not totally soluble in 0.01 M Tris, pH 8.0, as described by Bonner et al (1) for other chromatins; and upon shearing this solubility was not increased. It is also recognized by the absorption spectrum of SGC (Fig. 1) that a high degree of scattering is present. These observations might suggest that SGC is simply insoluble, and hence it cannot be transcribed in vitro. However, it will be shown that this is not the case.

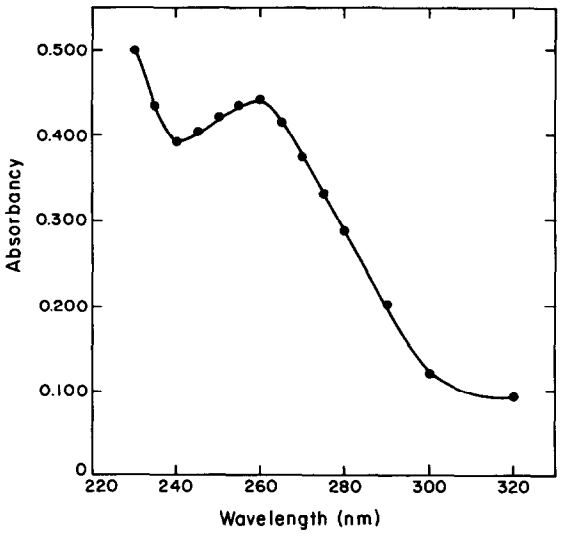

Fig. 1. The absorption spectrum of salivary gland chromatin in 0.01 M Tris buffer, pH 8.0, containing 0.001 M $MgCl_2$.

Chemical analysis, shown in Table 1, does not reveal any unusual protein DNA ratios, as observed with other chromatins (1). Disc gel electrophoresis shows a histone-DNA complimentarity similar to those found for liver chromatin. SGC was totally soluble in 2.0 M NaCl and in 5 M urea - 2.0 M NaCl as shown for other chromatins (7), and can be reconstituted to its native form by the method of Bekhor et al (7).

However, when NaCl or KCl was added to the assay mixture of SGC enhancement in RNA synthesis was observed. Table II shows the effect of NaCl on the template activity of SGC as compared to protein-free DNA and to liver chromatin. These results indicate that some stimulation is observed in the synthesis of RNA from protein-free DNA and liver chromatin, in agreement with DeBellis et al (14), yet absolute requirement for NaCl (or KCl) is seen for SGC. Figure 2 shows the effect of various concentrations of NaCl (or KCl) on the template activity of SGC (the concentration of 200 mM is chosen as optimum). That this may be due to salt participation in the transcription reaction is seen in the fact that when SGC is suspended in 200 mM NaCl, pelleted through 200 mM NaCl, and finally washed with 0.01 M Tris, pH 8.0,

TABLE I

CHEMICAL COMPOSITION OF SALIVARY GLAND CHROMATIN

Component	Mass Ratio*
DNA	1.00
RNA (Total)	0.125
RNA (RNase resistant)	0.056
Histone	1.14
Non-Histone Protein	1.17
Ribonuclease	0.00
Polysaccharides (Hexosamine)**	0.105
Glycoproteins (Sialic Acid)**	0.041

*Average of four determinations.
**Polysaccharides were determined by P. Benya, and glycoproteins by M. Golditch, graduate students of this department.

TABLE II

EFFECT OF NaCl ON THE ACTIVITY OF VARIOUS TEMPLATES

Template	Activity Relative to DNA in the presence of No NaCl	200 mM NaCl
DNA	88	100
Liver Chromatin	19.10	23.40
Salivary Gland Chromatin	0.00	13.35
0.6 M NaCl extracted Salivary Gland Chromatin	0	23.30

the pellet exhibits a similar absolute NaCl requirement for activity. Thus 200 mM NaCl is not dissociating proteins that may be responsible for the inactivity of SGC in the absence of NaCl. Extracting SGC with 600 mM NaCl does not change appreciably its monovalent ion requirement for genome

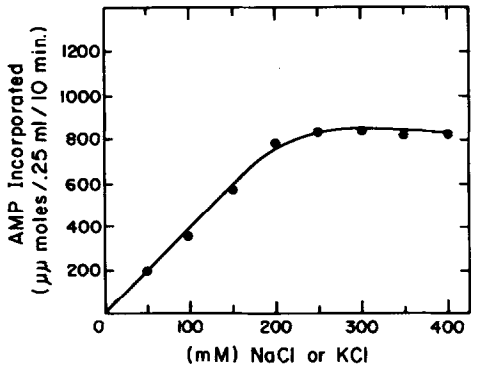

Fig. 2. The effect of various concentrations of NaCl (or KCl) on the template activity of salivary gland chromatin. The reaction mixture contained in addition to NaCl (or KCl) 10 μmoles Tris-HCl, pH 8.0; 1.0 μmole $MgCl_2$; 0.25 μmole $MnCl_2$; 3.0 μmoles mercapto-ethanol; 0.1 μmole each of GTP, CTP and UTP; 0.1 μmole ATP-^{14}C (specific activity, 1 μc/μmole); template up to 50 μg; E. coli RNA polymerase, 0.1-0.2 $O.D._{280nm}$; and deionized water to a final volume of 0.25 ml.

transcription, although at saturation the portion of DNA that is being read is increased over that of native chromatin (Table II). A comparison of the template activities of protein-free DNA, liver chromatin and SGC in a reaction mixture containing 200 mM NaCl is shown in Figure 3. Under these conditions the template activity of SGC approaches about 13% of that of protein-free DNA.

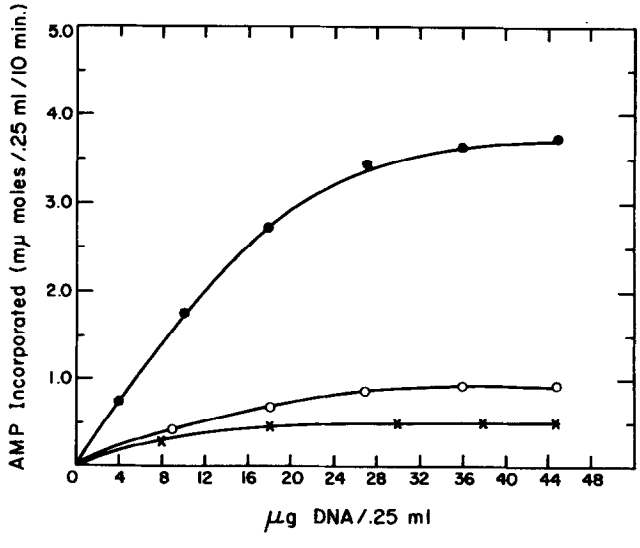

Fig. 3. Comparison of the template activities of protein-free DNA (●—●), liver chromatin (O—O), and SGC (X——X) in the presence of 200mM NaCl in a reaction mixture as described under Fig. 2.

The contents of polysaccharides and glycoproteins are also indicated in Table 1, as submaxillary glands specialize in the production of mucoproteins. That these contaminants in chromatin do not inhibit the transcription reaction was shown by incubating protein-free DNA and RNA polymerase in the presence and absence of SGC. This resulted in no inhibition in the incorporation of AMP into RNA transcribed from DNA by the addition of SGC.

Previously, KCl has been implicated in genome activation and deactivation (15). It was suggested that the influx of KCl in nuclei is directly related to puff formation, and thus to the turning on of the gene. The experimental data reported herein suggest that chromatin transcription, at least in this case, requires the participation of monovalent ions. Therefore, the template activity of a chromatin in general should also be examined in reaction mixtures utilyzing NaCl or KCl as described above.

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