A DNA-LIPID PROTEIN CONTAINING MATERIAL ISOLATED FROM CALF THYMUS NUCLEAR CHROMATIN

Vaughn Jackson, Joanne Earnhardt and Roger Chalkley

Department of Biochemistry
University of Iowa
Iowa City, Iowa 52240

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The bulk of the isolated nuclear chromosomal material, which is normally composed of rapidly sedimenting particles even though the DNA present is of relatively low molecular weight, can be obtained in a soluble form if a low ionic strength solution of chromatin* is sheared (Bonner et al., 1968; Zubay and Doty, 1959). However, a small limiting fraction of the nucleoprotein is insoluble even after extended shearing. We have inquired why this small fraction should be so resistant to solubilization by shearing. In this paper we shall show that the insoluble material consists of nucleohistone associated with a lipoprotein, a preliminary characterization of which is described.

MATERIALS AND METHODS

Calf thymus (ca. 8 gm) was homogenized (Waring blender) in 200 ml of sucrose-EDTA (0.25 M sucrose, 0.01 M Tris-HCl, 0.0016 M EDTA, 0.005 M $NaHSO_3$,

*The nomenclature used will be essentially that adopted by Bonner and his associates (Bonner et al., 1968). The term nucleoprotein refers of course to a complex of nucleic acid and protein, however, in the use of the term in this paper we shall imply specifically a complex of DNA and protein. Chromatin refers to the nucleoprotein complex of high S value isolated by gentle disruption of nuclei. Nucleohistone specifically refers to the soluble nucleoprotein of much lower S value obtained usually by vigorously shearing chromatin. A detailed description of the nomenclature and methods of isolation are described in a recent review by Bonner et al. (1968).

pH 8.0) for 90 seconds/30 V. The homogenate was strained through four layers of cheesecloth and through two layers of Miracloth (Chicopee Mfg. Co., Miltown, N.J.) and nuclear material was isolated by centrifugation (600 x g/20 min, SS-34 rotor). The pellet was successively washed by suspending and sedimenting from saline-EDTA (0.008 M EDTA, 0.25 M NaCl, 0.005 M $NaHSO_3$, pH 8.0). The final pellet was suspended in a Tris HCl-sodium bisulfite buffer (0.01 M Tris, 0.005 M $NaHSO_3$, pH 8.0); this material is described as chromatin. It was diluted with glass-distilled water to a final ionic strength of 5×10^{-4} M and sheared at maximum voltage in a Virtis homogenizer. The ionic strength was raised to 0.01 M and the solution centrifuged at 10,000 rpm/20 min. The insoluble material in the pellet was suspended in 0.01 M tris·HCl, pH 8.0 and washed by repeated centrifugation.

Lipid was isolated by repeated extraction with either n-butanol or chloroform-methanol (2:1). Protein and DNA were determined by the methods of Lowry et al., 1953, and Burton, 1956, respectively.

RESULTS AND DISCUSSION

Calf thymus chromatin can be sheared to a limiting extent as shown in Figure 1. It is seen that after three minutes no further yield of soluble nucleohistone is obtained. Under these conditions of vigorous shear approximately 1% of the DNA remains in an insoluble form.

Analysis of the insoluble fraction shows that it contains DNA, RNA protein and lipid in the mass proportions shown in Table I. While the lipid: protein mass ratio is similar in different preparations, we do not expect the DNA content to be constant as it is a function of the extent of shearing. Experiments currently in progress indicate that by appropriately adjusting the shear rate we can isolate an insoluble residue with a desired DNA content.

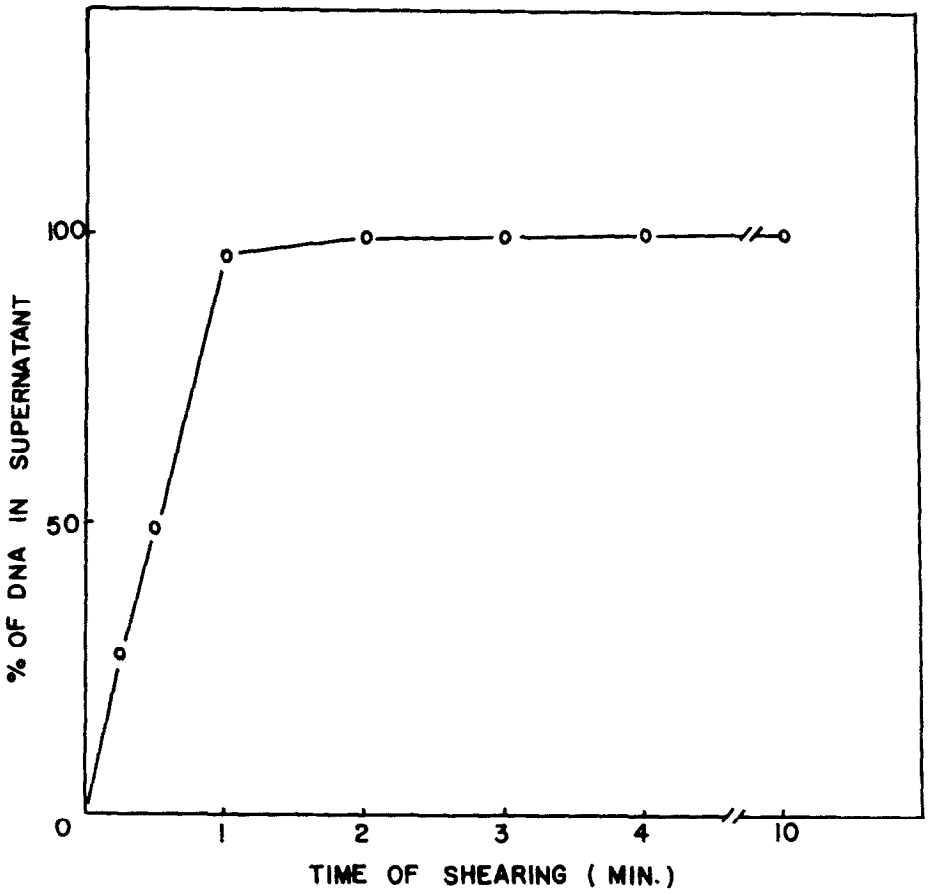

Fig. 1. Solubilization of chromatin by shearing. Calf thymus chromatin was sheared at maximum speed in a Virtis homogenizer. Aliquots were withdrawn at the appropriate time and sedimented (10,000 rpm/20') and the DNA content of the supernatant determined. Initial concentration of DNA in chromatin was 300 μg/ml.

TABLE I

Typical Composition of Insoluble Residue Isolated from Chromatin

Protein	Lipid	DNA	RNA
30.1 mg	9.0 mg	2.1 mg	0.4 mg

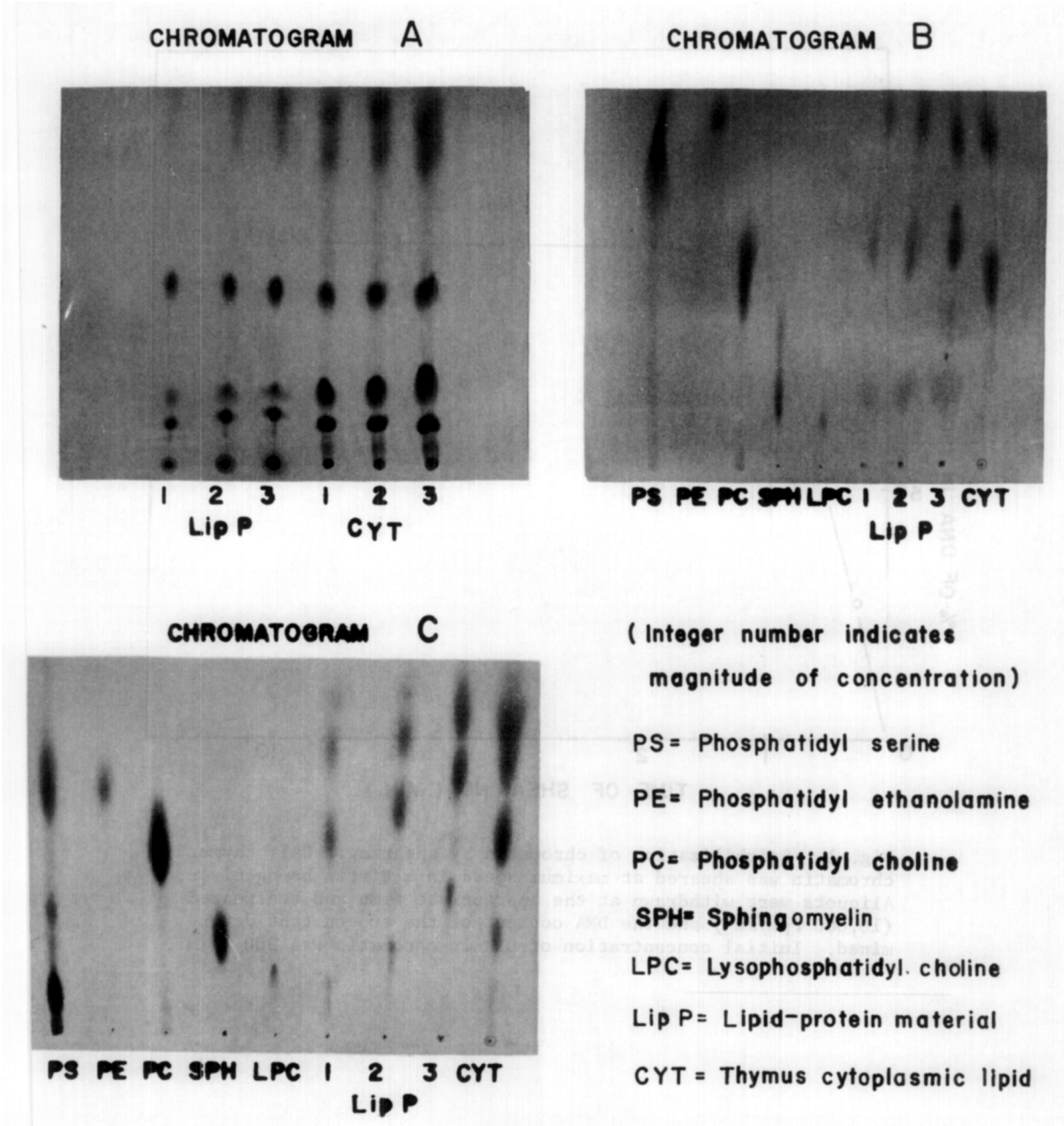

Fig. 2. Thin-layer chromatography of neutral lipids and phospholipids isolated from the insoluble residue. The stationary phase was silica gel G. Chromatogram A - neutral lipids separated by the solvents n-heptane, ether, acetic acid (80:20:1). Chromatogram B - phospholipids separated by the solvents chloroform, methanol, acetic acid, water (50:25:7:3). Chromatogram C - phospholipids separated by the solvents chloroform, methanol, ammonium hydroxide, water (75:30:4:0.5) (Nutter and Privett, 1968). The chromatograms were developed with phosphomolybdic acid (5%) in ethanol.

The nature of the lipid and protein component of the insoluble residue was examined. Lipid was repeatedly extracted with chloroform-methanol and found to contain both neutral and phospholipids as shown in Fig. 2. The major phosphatides found were phosphatidyl choline and phosphatidyl ethanolamine as shown in Chromatograms B and C. Gurr, Freeman and Hawthorne (1963) have shown that the bulk of the phospholipids extracted from intact liver nuclei consist of phosphatidyl choline and phosphatidyl ethanolamine. In addition, a brief statement should be made concerning the presence of heretofore unidentified phospholipids as shown in Chromatogram C. These phospholipids are present in minor quantities and different percentages than that of cytoplasmic lipid. It is of interest to note that this difference does exist. For amino acid analysis the insoluble residue was extracted with 0.4 N H_2SO_4 to remove histone, with organic solvents to remove lipids, and heated with 0.5 N perchloric acid to destroy nucleic acids. The amino acid composition of the residual protein is shown in Table II. If the bulk of the acidic amino acids are in the form of asparagine or glutamine as is evidenced by the large yield of ammonia upon acid hydrolysis, the overall charge on the protein is low, and this observation coupled with the high content of hydrophobic amino acid residues provides a reasonable explanation for the insolubility and the lipid-binding characteristics of this protein.

We then examined the possibility that the chromatin preparation was merely contaminated with cell membranes not directly associated with the chromatin itself. The chromatin solution was dissolved by lowering the ionic strength and sedimented through 2.0 M sucrose (ρ = 1.26 g/cc) which has a density sufficiently high to float cell membranes not firmly associated with the more dense nucleoprotein. The material which sedimented through the high density sucrose solutions contained lipoprotein and specific lipids in similar proportions to that described above for chromatin which had not been so treated.

TABLE II

Amino Acid Composition of Protein Component

Amino Acid	mole %
Aspartic acid	9.13
Glutamic acid	12.27
Threonine	5.42
Serine	6.27
Proline	5.146
Glycine	7.065
Alanine	7.75
Valine	6.024
Methionine	1.861
Isoleucine	4.499
Leucine	10.127
Cystine	0
Cysteic acid	0.841
Tyrosine	2.860
Phenylalanine	4.238
Lysine	7.329
Arginine	5.953
Histidine	3.206
Ammonia	11.6

Two important questions arise from the observations we have described. (1) Is the isolated nucleohistone-lipoprotein complex biologically meaningful and not simply generated during the disruption of the cells? The answer to this question will most likely be deduced from a study of the nature of the interaction between the components. (2) What is the nature of the lipoprotein? The lipid composition is not incompatible in the concept that it was initially part of a membrane system and this raises the possibility that it is in fact a fragment of the nuclear envelope. A number of reports based on electron microscopic evidence attest to the strong possibility of there being an interaction between chromatin and the nuclear membrane (Davies and Small, 1968; Duprau, 1965). This leads to a final question: What could be the role of such interactions? One possibility, which is supported by the recent observations of Comings and Kakefuda (1968), is that such an interaction is necessary for DNA synthesis in the mammalian cell, an observation analogous to the proposed dependence of DNA synthesis upon DNA-membrane interactions in bacterial systems (Smith and Hanawalt, 1967; Jacob et al., 1963).

Finally, the possibility that chromatin contains a substantial amount of nucleohistone linked to membranes (prior to the bulk of the nucleohistone being removed by shear) provides a feasible model to solve the problem of why isolated chromatin has a sedimentation constant higher by several orders of magnitude than the DNA contained within it.

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