# RNA ASSOCIATED WITH NONHISTONE CHROMOSOMAL PROTEINS OF DOG LIVER.

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Received July 24, 1973

SUMMARY: The nonhistone chromosomal proteins were separated on Sephadex G-200 into 3 fractions of which two were associated with 3S RNA. The RNA eluted with fraction I (guanine + cytosine content 54%) is tightly bound to the proteins from which it can be separated only after digestion with pronase. The RNA associated with fraction III (guanine + cytosine content 64%) can be separated from the proteins directly by chromatography on DEAE-Sephadex A 25. No dihydropyrimidines have been detected in any of the two RNAs.

The NHC proteins* are implicated in the regulation of gene expression. Preparations of these proteins usually contain tightly associated low molecular weight RNA (1). These RNAs have been found associated with NHC proteins isolated from a variety of tissues and species (2,3,4,5) and are sometimes called chromosomal RNAs. All these RNAs have been found to be similar in size but have been described as widely different in base composition, either containing or lacking dihydropyrimidines. In the present communication we describe the isolation and characterization of two low molecular weight species of RNA associated with dog liver NHC proteins.

## MATERIAL AND METHODS

Preparation of NHC proteins: Immediately after the sacrifice of the dogs the nuclei, chromatin and NHC proteins were prepared from the liver according to the method of Wang (1), with the modification that before the extraction of chromatin the nuclear sap proteins were removed by four 20 minutes extractions of nuclei, two with 0.14M NaCl, 0.01M Na citrate and two with 0.1M Tris-HCl buffer pH 7.6. The NHC proteins were released from the chromatin dissolved in 1M NaCl by lowering the NaCl concentration to 0.14M (1). The precipitated DNA-histones were removed by centrifugation (12,000 x g, 10 minutes) and the NHC proteins precipitated from the

*Abbreviation: NHC proteins = nonhistone chromosomal proteins.

supernatant by two volumes of cold acetone. The precipitate was collected the next day and washed twice with acetone and once with ether followed by drying under vacuum. All steps through the preparation of NHC proteins were carried out at 0-4°.

Fractionation of NHC proteins: The NHC proteins were solubilized over-night in solubilization buffer (0.02M Tris, 4M urea, 0.005M β-mercaptoethanol, 0.002M $Na_2$-EDTA and 0.1% sodium dodecylsulphate, pH 8.5) and fractionated on Sephadex G-200 column. The separated fractions were concentrated using aquacide (Calbiochem) and dialyzed against 0.01M Tris-HCl buffer, pH 8.5 for 48 hours. The fractions I and II were precipitated with 2 volumes of ethanol in the presence 2% potassium acetate. The fraction II was precipitated with 2 volumes of acetone.

Isolation of RNA by DEAE-Sephadex column chromatography: The separation of RNA from fractions I and III was carried out on DEAE-Sephadex A 25 columns (3). Fraction I was dissolved in solubilization buffer and dialyzed against 0.01M Tris-HCl, pH 8.0 (3 x 1000 ml) for 48 hours. It was then mixed with 2 ml pronase solution (5 mg/ml-preincubated at 37° for 90 minutes in 0.01M Tris-HCl, pH 8.0) and incubated at 37° for 8 hours. The RNA was precipitated with two volumes of ethanol in the presence of 2% potassium acetate. The precipitates were dissolved in 0.01 M Tris-HCl, pH 8.0 containing 0.2M NaCl and the solution was subjected to DEAE-Sephadex column chromatography. Fraction III was dissolved in the starting buffer and applied directly on DEAE-Sephadex column.

Analytical methods: The protein was determined by Lowry's procedure (6). DNA was assayed by the diphenylamine method (7) and RNA by the orcinol method (8). Base compositions of the RNA species were determined by paper electrophoresis (9) and column chromatography on Dowex-1 X8 (10). The RNA species were assayed for the presence of dihydropyrimidines by analyses for β-alanine in alkaline hydrolysates of RNA (11), by Fink's color reaction with p-dimethylaminobenzaldehyde (12), and by the colorimetric method of Ceriotti and Spandrio (13) after the precipitation of RNA with cetyltrimethylammonium bromide (14). Polyacrylamide gel electrophoresis of RNA was carried out in 0.5 x 6 cm 8% gel according to

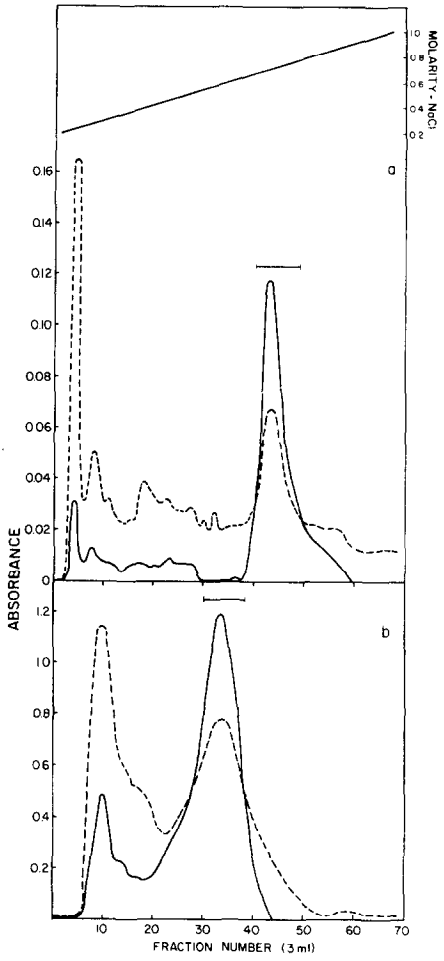


Fig. 1. Elution patterns of (a) fraction I RNA and (b) fraction III RNA. The RNAs were eluted from 0.9 cm x 30 cm columns of DEAE-Sephadex A-25 by 200 ml of a linear NaCl gradient (0.2 to 1.0 M) in 0.01 M Tris-HCl, pH 8.0. The flow rate was 3.0 ml/hr.

Loening (15) and electrophoresis of NHC proteins in 6% gel according to Fambrough et al. (16).

## RESULTS

RNA content in NHC proteins: The NHC proteins prepared from dog liver nuclei contain 6.03 ± 0.7% of RNA (average from 5 isolations). The preparations were devoid of DNA and polyacrylamide gel electrophoresis at pH 4.5 has shown only traces of histones. The RNA content of various preparations was fairly constant and the mass ratio of RNA/protein varied from 0.053 to 0.077. The UV-absorp-

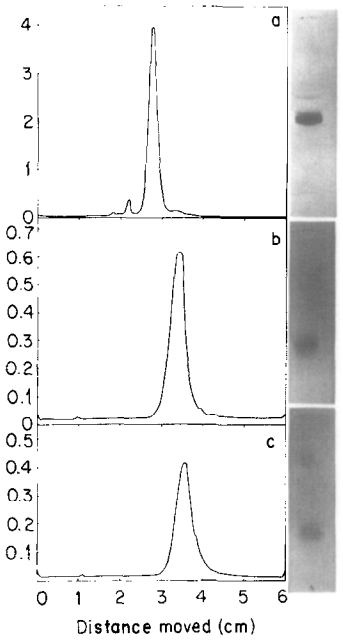


Fig. 2. Polyacrylamide gel electrophoresis of (a) yeast tRNA, (b) fraction I RNA and (c) fraction III RNA. The gels were stained with methylene blue and scanned at 600 nm in a Gilford spectrophotometer.

tion spectrum of NHC proteins in solubilization buffer, pH 8.5 showed an absorption maximum at 264 nm and the average ratio of absorption at 280 nm/260 nm was 0.830.

Gel filtration of NHC proteins on Sephadex G-200 column: The NHC proteins separated into three main fractions. The analysis of these fractions is given in Table 1. The fraction I having an RNA/protein mass ratio of 0.215 elutes in the void volume of the column and represents the high molecular weight components of NHC proteins. This fraction exhibits the UV-absorption spectrum of a nucleoprotein with the absorption maximum at 260 nm and high absorption at 230 nm. The fraction II consists mainly of proteins with traces of RNA (may be due to contamination from adjacent fractions) and has an RNA/protein mass ration of 0.02 and shows absorption maximum at 260 nm and absorption minimum at 232 nm.

Characterization of RNA components of fractions I and III: The RNA components of both fractions were isolated on DEAE-Sephadex columns. To separate RNA from the proteins in fraction I it was necessary to digest the NHC proteins by

TABLE 1. Analysis of Fractions of NHC Proteins Separated on Sephadex G-200 Column.

| FRACTION | EXPERIMENT NO. | 280 nm/260 nm | PROTEIN (mg) | RNA (mg) |
|---|---|---|---|---|
| I | 1 | 0.745 | 100 | 21.0 |
| | 2 | 0.702 | 100 | 22.0 |
| II | 1 | 1.210 | 100 | 2.0 |
| | 2 | 1.430 | 100 | 1.8 |
| III | 1 | 0.552 | 100 | 217.0 |
| | 2 | 0.546 | 100 | 200.0 |
| | 3 | 0.527 | 100 | 207.0 |
| | 4 | 0.560 | 100 | 195.0 |

40-50 mg of protein in 8-10 ml of solubilization buffer was loaded on 2.5 x 45.0 cm column and eluted with the same buffer at a rate of 6-10 ml per hour. 95% of the UV absorbing material was recovered from the column.

TABLE 2. Nucleotide Composition of Fraction I and Fraction III RNA

| | C | A | U | G | $\frac{A+U}{G+C}$ | $\frac{A+G}{U+C}$ |
|---|---|---|---|---|---|---|
| Fraction I | 20 | 20 | 26 | 34 | 0.85 | 1.17 |
| Fraction III | 28 | 12 | 24 | 36 | 0.56 | 0.92 |

The base composition is an average from three determinations performed on different preparations. RNAs from two preparations were analyzed by paper electrophoresis and from one preparation by column chromatography.

pronase. In fraction III it was possible to separate RNA from the proteins without previous enzymatic hydrolysis of the proteins. The elution profiles (Fig. 1) of the DEAE-Sephadex column chromatography show that the RNA components of fraction I is eluted with 0.7 M NaCl and that of fraction III with 0.6 M NaCl. The protein part of fraction III was eluted with 0.25-0.35 M NaCl. The two species of RNA so purified exhibit characteristic UV-absorption spectra of a nucleic acid. They are hydrolyzed after heating with 0.1 M KOH at 100°C for 20 minutes and give a positive orcinol reaction.

The base compositions of the two types of RNA isolated from fractions I and III are presented in Table 2. Both RNAs are rich in guanine and the ratio of A+U/

G+C is higher for fraction I RNA. The purine/pyrimidine (A+G/U+C) ratio for fraction I RNA is 1.17 and that for fraction III RNA is 0.92. Both species of RNA gave negative Fink's color reaction (12) and after alkaline hydrolysis no ninhydrine positive spot on a paper chromatogram which would correspond to β-alanine (11) was detected. The used methods could detect less than 0.5 mole percent of the dihydropyrimidines in the analyzed RNAs. In simultaneously analyzed yeast tRNA (Miles Lab., Inc.) 3.6 mole percent of dihydrouridylic acid were determined. These results closely correspond to values given for yeast tRNA by Margrath and Shaw (11). These results clearly indicate the absence of dihydropyrimidine in both the species of RNA isolated from NHC-protein fractions. On polyacrylamide gel both RNAs move as a single broad band with almost the same mobility (Fig. 2). Calculations based on the migration of yeast t-RNA (4S) and its contaminant (5S) RNA show that both have S value around 3S and approximate molecular weight of 13,000 corresponding to an average chain lengths of 40 to 50 nucleotides.

## DISCUSSION

We have described two types of 3S RNA one firmly and one losely associated with NHC proteins which differ from each other in base composition and both lack dihydropyrimidines. In size and base composition our fraction I RNA is identical with the RNA isolated by Getz and Saunders by phenol extraction of the total chromatin of human lymphocytes (5). Our fraction III RNA differs by its nucleotides composition from all other chromatin-associated RNAs described in the literature. It is nearly identical in size and composition with the calf thymus chromosomal RNA described by Shih and Bonner (2). From this RNA it differs only by the absence of dihydrouracil. The amount of uridylic acid in our fraction III RNA is equal to the content of uridylic and dihydrouridylic acid in the calf thymus chromosomal RNA. An artefactual origin of the described fraction I and fraction III RNAs is not probable. The amount of RNAs associated with NHC proteins of the dog liver, their size and base composition have been constant from preparation to preparation. Their base composition differ significantly from the base composition of ribosomal

RNA (3), transfer RNA (3) or heterogeneous nuclear RNA (5). Therefore our RNAs could not have originated by a random degradation of these types of cellular RNA. This conclusion is supported by the findings of Getz and Saunders (5) who after studying the hybridization properties of the 3S RNA isolated from the chromatin of human leukocytes excluded an artifactual origin of the chromatin-associated RNA. The proposal of Heyden and Zachau (17) that the chromatin-associated RNA is a degradation product of transfer RNA also disagrees with the absence of dihydrouridylic acid in our RNA preparations.

Acknowledgement: This work was supported by a grant from The Robert A. Welch Foundation, N. H 393.

## REFERENCES

1. Wang, T. Y. (1967) J. Biol. Chem. 242, 1220-1226.
2. Shih, T. Y., and Bonner, J. (1969) Biochim. Biophys. Acta 182, 30-35.
3. Dahmus, M. E., and McConnell, D. J. (1969) Biochemistry 8, 1524-1534.
4. Arnold, E. A., and Young, K. E. (1972) Biochim. Biophys. Acta 269, 252-258.
5. Getz, M. J., and Saunders, G. F. (1973) Biochim. Biophys. Acta 312, 555-573.
6. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) J. Biol. Chem. 193, 265-275.
7. Burton, K. (1956) Biochem. J. 62, 315-323.
8. Ceriotti, G. (1955) J. Biol. Chem. 214, 59-70.
9. Markham, R., and Smith, J. D. (1952) Biochem. J. 52, 552-557.
10. Volkin, E., and Carter, C. E. (1951) J. Am. Chem. Soc. 73, 1516-1519.
11. Magrath, D.I., and Shaw, D. C. (1967) Biochem. Biophys. Res. Commun. 26, 32-37.
12. Fink, R. M., Cline, R. E., McGaughey, C., and Fink, K. (1956) Anal. Chem. 28, 4-6.
13. Ceriotti, G., and Spandrio, L. (1963) Clin. Chim. Acta 8, 295-299.
14. Sibatani, A. (1970) Anal. Biochem. 33, 279-285.
15. Loening, U. E. (1967) Biochem. J. 102, 251-257.
16. Fambrough, D. M., Fujimura, F., and Bonner, J. (1968) Biochemistry 7, 575-584.
17. Heyden, H. W. V., and Zachau, H. G. (1971) Biochim. Biophys. Acta 232, 651-660.