THE ABSENCE OF SATURATED PYRIMIDINE BASES IN CHROMATIN-ASSOCIATED RNA FROM AVIAN RETICULOCYTES AND MOUSE ASCITES CELLS

P. Tolstoshev and J.R.E. Wells

Department of Biochemistry, University of Adelaide, Adelaide, South Australia 5001.

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Summary

Low molecular weight RNA (3-4S) has been isolated from the chromatin of avian reticulocytes and mouse ascites cells by the procedures used to isolated chromosomal RNA*. Although dihydrouridine was readily detected in tRNA, neither this base, nor dihydroribothymidine, was found in detectable levels in chromatin-associated RNA or ribosomal RNA. The presence of saturated pyrimidines is not an invariant property of chromosomal RNA.

INTRODUCTION

Chromosomal RNA, a low molecular weight RNA species isolated from the chromatin of many cell types (1,2,3) has been implicated in eukaryotic gene regulation (4). Among the unique properties ascribed to this RNA species is a high content (8 - 10 moles per cent) of a saturated pyrimidine, either dihydrouridine or dihydroribothymidine (5). Recent reports have suggested that cRNA may arise from the degradation of tRNA (6) or from rRNA (7,8). We have measured the levels of saturated pyrimidine bases in tRNA (yeast, avian reticulocyte), in rRNA (avian reticulocyte), and in cRNA preparations from avian reticulocytes and mouse ascites cells; first, to examine whether these unusual bases are an invariant property of cRNA and secondly, to investigate the possible origins of cRNA.

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*As defined by the preparative procedures of Dahmus and McConnell (2).
Abbreviations - cRNA = chromosomal RNA; tRNA = transfer RNA; rRNA = ribosomal RNA.

MATERIALS AND METHODS

Cells.

Reticulocytes (chiefly mid and late polychromatic erythrocytes) were obtained from the circulation of hens made highly anaemic by injection with phenylhydrazine (9). Ehrlich mouse ascites cells were serially passaged through male albino mice, were harvested at day 6 - 7, and were washed as described by Dahmus and McConnell (2).

Preparation of Chromatin.

Reticulocyte chromatin was prepared as described by Appels *et al.* (9), and mouse ascites chromatin by the method of Dahmus and McConnell (2). In both cases, the preparation prior to centrifugation through 1.7M sucrose (i.e., crude chromatin) was used as the starting material for the preparation of cRNA.

Preparation of Chromosomal RNA.

This was exactly as described by Dahmus and McConnell (2) involving centrifugation of chromatin through 4M CsCl, pronase digestion of the protein skins, phenol extraction and chromatography through DEAE-Sephadex. These and other RNA preparations were further characterized by sucrose gradient centrifugation (Fig. 1).

Estimation of Dihydrouridine and Dihydroribothymidine Contents of RNA Samples.

The positive uriedo reaction (10) of saturated pyrimidines has been used to estimate the levels of these bases in cRNA preparations (4,11,12). We found great difficulty in removing the last traces of urea from cRNA isolated after chromatography in 7M urea (see also ref.12), and since urea also gives a positive uriedo reaction, we measured the specific amino acid hydrolysis products of the two saturated pyrimidines.

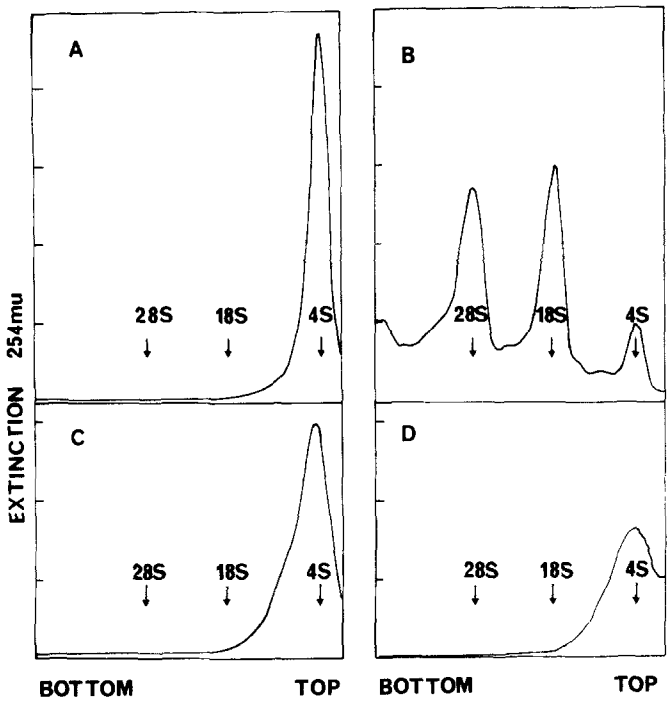

FIGURE 1. Sucrose density gradient analysis of RNA samples. The samples were analysed on linear 5-20% sucrose gradients as described in the text. A - reticulocyte tRNA; B - reticulocyte rRNA, C - reticulocyte cRNA; D - mouse ascites cRNA.

RNA samples were hydrolysed with 0.2N NaOH as described by McGrath and Shaw (13). The specific hydrolysis products, β-alanine from dihydrouridine, and β-amino isobutyric acid from dihydroribothymidine, were estimated on a Beckman Model 120C Amino Acid Analyser fitted with a 10-fold scale expander. A 60 cm column was packed to a height of 27 cm with resin, and 1.0 ml samples (in 0.1N HCl containing 0.02 μmoles/ml of nor-leucine) loaded. Runs started at 32.5°C with standard pH 3.25 citrate buffer, with an increase to 60° at 45 min. and a buffer change to pH 4.30 at 80 min. β-alanine and β-amino isobutyric acid were clearly resolved from all other amino acids, and eluted as sharp peaks at 151 and 158 minutes respectively (Fig. 2). Standards of the two amino acids co-chromatographed with RNA hydrolysates

eluted in the expected positions. The recovery of β-alanine from the hydrolysis of dihydrouridine was 67.1% (mean of three estimates) in close agreement with the estimate of McGrath and Shaw (13) and the recovery of β-amino isobutyric acid from dihydroribothymidine hydrolysis was 66.2% (mean of three estimates). The content of unusual bases in the RNA samples was calculated as moles %, assuming an average nucleotide residue weight of 320, and making corrections for the recovery of the hydrolysis products.

Preparation of Other RNA Samples.

Yeast tRNA was extracted by the method of Holley *et al.* (14). Reticulocyte tRNA was isolated by phenol extraction of the high-speed supernatant of a reticulocyte lysate, and was further purified by DEAE-cellulose chromatography (14). Reticulocyte rRNA was obtained by phenol extraction of a polysome preparation from lysed red cells, and, when analysed on 5-20% sucrose gradients, contained predominantly 18 and 28S material, with a small 4-5S component (see Fig. 1).

Sucrose Gradient Analysis.

40-120 μgm of the RNA samples were loaded onto linear 5-20% sucrose gradients (sucrose gradient buffer - 5mM MgAc, 10mM Tris, 150mM NaCl pH 7.4), centrifuged 41K, 4 hr., 3°C in the SW41 rotor of a Beckman L_2 Ultracentrifuge, and were fractionated through an LKB flow cell spectrophotometer. The extinction at 254 mμ was monitored on a recorder.

Synthesis of dihydroribothymidine.

This was prepared from ribothymidine (Calbiochem, A grade) by the method of Cohn and Doherty (15).

Other Analytical Procedures.

Protein was estimated by the method of Lowry *et al.*

(16), DNA by the diphenylalanine reaction (17) and RNA by the orcinol method (18). For chromatin samples, the orcinol reaction was performed on the perchloric acid supernatant after alkaline hydrolysis (19).

RESULTS AND DISCUSSION

Table I shows the chemical composition of the chromatin preparations from both cell types investigated. The percentage of total chromatin-associated RNA recovered as cRNA was highly variable, but averaged 20% for reticulocytes and 24% for the mouse ascites cells. The variability is due to the variable amount of total RNA in the chromatin which remains associated with the skin of protein floating on 4M CsCl. The amount in the skin ranged from 40-70% of the total RNA for ascites chromatin, and from 40-80% for reticulocyte chromatin. This is consistent with previous observations (7).

The cRNA from both cell types eluted as a sharp peak at an NaCl concentration of about 0.55M on DEAE-Sephadex chromatography, and both cRNA's had a mean sedimentation value, as

TABLE I

CHEMICAL COMPOSITION OF AVIAN RETICULOCYTE AND MOUSE ASCITES CHROMATIN

	Protein	RNA	DNA
Reticulocyte n = 6	3.328 ±0.162	0.219 ±0.021	1
Ascites n = 4	3.630 ±0.217	0.172 ±0.018	1

Chemical composition of crude chromatins, prepared as described in the text. Values, together with the S.E.M.'s, are expressed relative to the DNA content.

TABLE II

DIHYDROPYRIMIDINE CONTENT OF RNA SAMPLES

Sample	Dihydrouridine Content	Dihydroribothymidine Content
Yeast tRNA	4.63 ± 0.22 (6)	0 (6)
Reticulocyte tRNA	3.01 ± 0.26 (5)	0 (5)
Reticulocyte rRNA	trace (2)	0 (2)
Reticulocyte cRNA	0 (5)	0 (5)
Ascites cRNA	0 (4)	0 (4)

The dihydropyrimidine contents of various RNA samples were estimated as described in the text. The values, together with the S.E.M. are expressed as moles %. The figures in brackets indicate the number of estimations. The limits of detection ranged from 0.1 to 0.4 moles %, depending on the amount of RNA available for analysis.

estimated by sucrose density gradient centrifugation, of 3-4S. However, the size heterogeneity was considerably greater than for reticulocyte tRNA (see Fig. 1).

Figure 2 shows the position of elution of β-alanine and β amino isobutyric acid, on amino acid analysis as described in Materials and Methods. Beckman Amino Acid Calibration Mixture, and norleucine were also present in the sample loaded. β alanine and β amino isobutyric acid are clearly resolved from the other amino acids eluting in this region.

Table II shows the content of saturated pyrimidine bases of cRNA from reticulocytes and mouse ascites cells, as well as that of yeast and reticulocyte tRNA, and reticulocyte rRNA. Neither of the bases was detected in the cRNA samples (limits of detection 0.1 to 0.4 moles %) whereas the presence of dihydrouridine in tRNA was readily demonstrated.

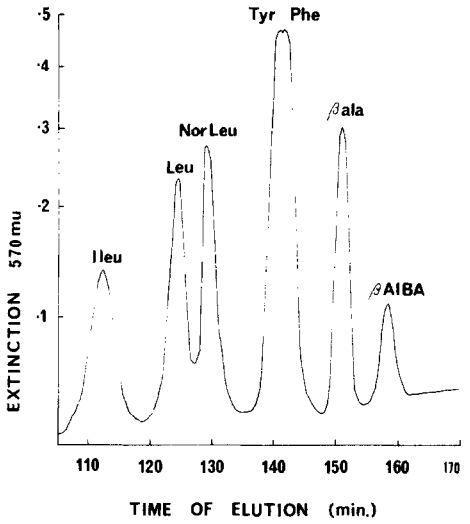

FIGURE 2. A portion of the amino acid elution profiles, showing the relative positions of β alanine (0.1 μmoles) β amino isobutyric acid (β AIBA, 0.025 μmoles) and the amino acids of Beckman Amino Acid Calibration Mixture, containing norleucine (0.02 μmoles of each amino acid).

The value for yeast tRNA was slightly higher than that of other reports (12,13). The trace of dihydrouridine found in rRNA may have arisen from contaminating tRNA (see Fig. 1,B).

The presence in cRNA of saturated pyrimidine bases, at levels not found in other RNA species, is strong evidence for its existence as an unique RNA species. However, the failure to find either of these bases in the cRNA preparations from reticulocytes and mouse ascites cells still allows the possibility (6,7,8) that this RNA derived from other cellular RNA species in these cells. The fact that tRNA contains dihydrouridine, whereas none was detected in cRNA, argues against the suggestion (6) that cRNA arises from tRNA degradation. However, results presented here are compatible with the notion (7,8) that for several chicken tissues, the major part of RNA associated with chromatin is derived from rRNA.

This report, together with others in which saturated

pyrimidine bases were not found in chromatin-associated RNA from sea-urchin embryos (20) or in cRNA isolated from rat liver (12) make it clear that the presence of these bases is not an invariant property of RNA species in chromatin.

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