Solubilization and Characterization of the Residual Proteins of the Cell Nucleus*

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SUMMARY

The nuclear residual proteins of rat liver have been solubilized by treatment with sodium deoxycholate and separated into four major fractions. These residual proteins are acidic, highly heterogeneous, and contain different amounts of ribonucleic and deoxyribonucleic acid. Their amino acid compositions show that these are distinctly different protein fractions. Furthermore, these residual acidic proteins form complexes with histones to various degrees. The possible significance of these characteristics is discussed.

When isolated cell nuclei are extracted exhaustively with Tris buffer or with dilute 0.9% NaCl and 1 to 2 M NaCl, a residual mass remains that resists extraction by any mild solvents. Unlike the histones which are basic, the residual fraction contains acidic protein or proteins. Mayer and Gulick (1), Wang et al. (2), Wang, Mayer, and Thomas (3), Dallam and Thomas (4), and Carver and Thomas (5) and later Engbring and Laskowski (6), with dilute alkali as an extractant, obtained an acidic protein from the nuclear residual protein fraction. This alkali-soluble protein, or acidic protein fraction, consists of lipids (3), ribonucleic acid (7–9), and deoxyribonucleic acid (8, 10) and, in distinction to the histones, protein containing tryptophan (11). It is present in significant amount in the cell nucleus, particularly in tumor cells (2, 10, 12). Furthermore, isotopic studies (7, 10, 13) have shown that the residual acidic protein (or proteins) exhibits a high rate of incorporation of isotopic activity.

Morphologically (14–16), the nuclear residual fraction consists of nucleoli, nuclear envelope, and structures resembling the "residual chromosomes" of Mirsky and Ris (17). The alkali-soluble protein prepared from the nuclear residual protein fraction is considered to correspond to nucleoli and residual chromatin (15). Postulations that the residual protein is associated with chromosome structure (18–20), is a part of the mitotic apparatus (21), and plays a role in conjunction with histones in the regulation of DNA synthesis (22) have also been made. These considerations, together with the characteristics summarized above, underline the importance of the nuclear residual proteins in the over-all biochemical activities of the cell nucleus.

The amino acid composition and NH₂-terminal residues of the residual acidic protein have been determined by Busch and Steele (23, 24) and by Dounce and Hilgartner (20). The results showed that the acidic proteins prepared from various sources have similar amino acid composition and, based on the number of NH₂-terminal residues, have a calculated average molecular weight of 80,000 to 400,000 (22).

In spite of its significance, relatively few studies have been made on the nuclear residual acidic protein, mainly because of its insolubility in physiological media. Furthermore, the use of alkali as the extractant has been considered too drastic. The possibility that the insolubility of the residual acidic protein may be due to its lipid content has been indicated in the work on calf thymus residual proteins (25, 26). A high molecular weight complex containing DNA, RNA, and protein has been isolated by digesting the nuclear residual fraction with DNase followed by sodium deoxycholate treatment (25). Such preparations incorporate labeled amino acids as actively as the nuclear residual fraction from which they are derived (27). The complex disperses easily in aqueous buffers, has the appearance of spherical particles associated with DNA-containing strands, and is partly precipitable at acidic pH (26). These results led to the present investigation in an attempt to solubilize and isolate the residual acidic protein by mild treatment. Such a procedure will be described in this report. It will be shown that nearly all the nuclear residual protein of rat liver can be solubilized by this procedure and that the acidic protein, which is highly heterogeneous, can be separated into four fractions. Results on the characteristics of these acidic proteins will be presented.

EXPERIMENTAL PROCEDURE

Male Sprague-Dawley rats weighing about 200 g were used. The animals were fasted for 24 hours and were killed by decapitation. The livers were perfused with ice-cold 0.9% NaCl. A 30% (w/v) liver homogenate was made in 0.25 M sucrose-0.003 M CaCl₂, with the use of a motor-driven Teflon homogenizer. About 30 to 40 strokes were required for a satisfactory homogenization. Microscopic examination of such homogenates showed less than 1% contamination by whole cells. The homogenate was filtered through two layers of nylon cloth, layered over 0.34 M sucrose (28) containing 0.003 M CaCl₂, and centrifuged at 2500 × g for 10 min. Sediment from this centrifugation was collected, rehomogenized in a Dounce homogenizer (28), and centrifuged in 0.003 M CaCl₂-2.2 M sucrose solution according to the method of Chauveau, Moulé, and Roueller.
Characterization of Residual Proteins of Cell Nucleus

Vol. 241, No. 12

Protein, RNA, and DNA content of nuclear residual fraction of rat liver

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Amount</th>
<th>Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Protein</td>
<td>RNA</td>
</tr>
<tr>
<td>Residual fraction before</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DOC treatment</td>
<td>69.6</td>
<td>8.0</td>
</tr>
<tr>
<td>Residual fraction solubilized by DOC*</td>
<td>66.6</td>
<td>6.5</td>
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</tbody>
</table>

* Clarified at 20,000 x g for 20 min.

Relative amounts of protein, RNA, and DNA of nuclear residual acidic proteins of rat liver

<table>
<thead>
<tr>
<th>Acidic protein fraction</th>
<th>Proportion of total solubilized residual fraction as</th>
<th>Protein</th>
<th>RNA</th>
<th>DNA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>%</td>
<td>%</td>
<td></td>
</tr>
<tr>
<td>R-RNP</td>
<td>4.1</td>
<td>2.8</td>
<td>27.7</td>
<td></td>
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<tr>
<td>pH 6</td>
<td>88.6</td>
<td>94.2</td>
<td>54.5</td>
<td></td>
</tr>
<tr>
<td>pH 5</td>
<td>4.3</td>
<td>1.2</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>(NH₄)₂SO₄ ppt.</td>
<td>3.0</td>
<td>1.8</td>
<td>3.9</td>
<td></td>
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</table>

* Average of four determinations.

RESULTS

The nuclear residual fraction contains about 90% protein and 10% RNA, with trace amounts of DNA (Table I). It can

6 supernatant to pH 5 precipitated the pH 5 fraction. This was again collected by centrifugation. The pH 5 supernatant was salted out by 0.5 saturated ammonium sulfate. The acidic proteins thus obtained were dissolved in 0.05 M Tris buffer, pH 8.5, and dialyzed overnight.

Total histone and DNA were prepared from calf thymus nucleohistone by extraction with 0.2 n HCl (22), and by the method of Kay, Simmons, and Dounce (31), respectively. The histone-HCl was dialyzed against distilled water and used without further treatment.

Protein concentration was determined by the method of Lowry et al. (32) with human serum as standard, or, in the case of histones, by weighing. DNA was measured by the procedure of Burton (33) and RNA by the orcinol method (34). Sedimentation patterns of the residual acidic proteins were obtained with a Spinco model E analytical ultracentrifuge and the calculated sedimentation coefficients were corrected to 20° in water. Amino acid analyses were carried out on acid hydrolysates of the protein samples prepared as described previously (35) and determined with a Spinco model 120 amino acid analyzer according to Moore, Spackman, and Stein (36). Tryptophan was assayed on separate protein samples according to the method of Spies and Chambers (37). Nucleotide composition of RNA was determined on alkaline hydrolysates (35) of the acidic protein samples by paper electrophoresis after the procedure of Markham and Smith (38).

TABLE II

<table>
<thead>
<tr>
<th>Acidic protein fraction</th>
<th>RNA</th>
<th>DNA</th>
</tr>
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<tbody>
<tr>
<td>R-RNP</td>
<td>0.56</td>
<td>0.08</td>
</tr>
<tr>
<td>pH 6</td>
<td>0.009</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>pH 5</td>
<td>0.23</td>
<td>0.004</td>
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<tr>
<td>(NH₄)₂SO₄ ppt.</td>
<td>0.51</td>
<td>0.002</td>
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</table>

* Average of four analyses.

The abbreviations used are: DOC, sodium deoxycholate; R-RNP, residual ribonucleoprotein particles.
be seen that DOC solubilizes 95% of the total rat liver nuclear residual protein, but only 80% of the residual RNA. Phenol extraction of the solubilized residual proteins gave an RNA preparation with a sedimentation coefficient of 4 S. DNA, while representing a very small fraction of the total residual nucleoprotein, is reduced by the same proportion as RNA after the detergent treatment.

When the solubilized nuclear residual protein was fractionated according to the scheme described under “Experimental Procedure,” four protein fractions were obtained with relative protein content shown in Table II. The values can only be considered approximate, since about 60% of the R-RNP pellet could not be brought back into buffer solution and is not included. All the studies were made on the soluble R-RNP. The insoluble portion of the R-RNP pellet resisted treatment by higher concentration of DOC, Triton X-100, and EDTA. It is, however, soluble in 4 M urea and 0.1 M NaOH. The other three protein fractions were freely soluble in buffer.

The R-RNP fraction is completely precipitable at pH 5 as shown in Fig. 1. It can also be seen that the separation of the pH 6 and pH 5 fractions is incomplete because of their wide pH precipitation range. After removing the pH 5 fraction from the DOC-treated solution, further acidification of the solution to pH 3 did not result in any measurable precipitation. Salting-out with 0.5 saturated ammonium sulfate, however, precipitated nearly all the protein remaining in the pH 5 supernatant.

The pH 6 fraction constitutes 89% of the total residual protein (Table II). The remainder is distributed equally among the other three residual protein fractions. These protein fractions have a different RNA content (Table III). The pH 6 fraction contains the least RNA (less than 1%); whereas one-third of the R-RNP and of the (NH₄)₂SO₄-precipitated fractions is RNA. The RNA content of the pH 5 fraction lies between that of the other two fractions, being 19%. The compositions of the major nucleotides of the RNA of these fractions also differ from each other, as shown in Table IV. The general characteristic of the RNA of these acidic proteins is their high (G + C):(A + U) ratios, which are about 2. The equimolar ratios of A:U and C:G of the pH 5 RNA may suggest some orderly configuration of the RNA structure.

The amino acid composition of the four residual acidic protein fractions is given in Table V. Values of the alkali-soluble protein fraction from the nuclear residual protein of rat liver obtained by Busch and Steele (24) and by Dounce and Hilgartner (20) are also included for comparison. The acidic nature of these proteins is apparent. The ratios of acidic to basic amino acid residues vary from 1.44 for the pH 5 fraction to 1.05 for the pH 6 fraction, as compared to ratios of 1.4 to 1.5 for the alkali-soluble proteins. The variation is mainly due to glutamic acid content. While the alkali-soluble protein has 12 to 13 mols % of glutamic acid, the acidic protein fractions have only 8 to 9 mols %. The pH 5 and the (NH₄)₂SO₄-precipitated fractions have lower arginine content than the R-RNP and the pH 6 fractions. Significantly higher tyrosine and alanine values were...
All the residual acidic proteins are highly heterogeneous in the analytical centrifuge, as shown in Fig. 2. Major components of the acidic proteins have sedimentation coefficients ranging from 2 to 7 S. Although the R-RNP was obtained from the 105,000 × g pellet, its sedimentation schlieren patterns show only minute amounts of 83 S and 26 S components. The major component (or components) of the R-RNP sedimented at 5.4 S. Since 60% of the pellet protein did not redisolve in the buffer, most of the high molecular weight component of the R-RNP must be present in the insoluble material. Because of their polydispersity, very little can be said at present with respect to the solution properties of these acidic proteins. Further fractionation of these proteins is intended.

The residual acidic proteins interact strongly with histones and form a complex. Upon mixing the acidic proteins with histones, instantaneous precipitation occurred. Labeled acidic proteins were prepared by incubation of the nuclear residue with 14C-tryptophan (27). These 14C-tryptophan-labeled proteins were used to test their precipitatibility with the histones. The results (Fig. 3) showed that the degree of precipitation varied with the individual acidic proteins. Of the four fractions, the pH 6 fraction interacted most strongly and was completely precipitated with histones. The other three acidic proteins, in decreasing order: pH 5, R-RNP, and (NH4)2SO4 precipitate fraction, interacted less with histones. In all cases, the amount of 14C-tryptophan-labeled acidic protein that has precipitated was proportional to the amount of the proteins added.

**Discussion**

The term “residual protein” requires some clarification. In the work of Steele and Busch (23) and Busch and Steele (24) and of the Russian workers (9, 14–16), “residual protein” represents the residue after cell nuclei have been successively extracted with 0.14 M NaCl or buffer or both (to remove nuclear ribosomes and soluble proteins), with 2 M NaCl (to remove DNA-histones and associated proteins), and with dilute NaOH (to remove the alkali-soluble proteins). The unusual presence of hydroxylysine, hydroxyproline, and the high glycine content in their residual proteins suggest collagen-like proteins and therefore this residual protein may consist mostly of nuclear envelope (23) as well as the proteins associated with the DNA-histones (19, 20). Douce and Hilgarter (20) used nuclear preparations which have been extracted with 0.14 M NaCl or 0.1 M HCl or both (to remove globulins and histones), with alcohol-ether mixture (to remove lipids), and by DNase digestion to remove DNA. Their residual proteins would thus include the alkali-soluble proteins as well as the proteins associated with the DNA-histones (19, 30). Allfrey, Daly, and Miersky (29) refer to the residual proteins as that fraction of the cell nuclei which remains after thorough extraction with 1 M NaCl. The nuclear residual proteins described in this paper correspond to those of Allfrey et al. and hence include the alkali-soluble proteins.

The large amount of the pH 6 fraction (88.8%) in the solubilized residual proteins is in agreement with the early reports (1–5) in which the acidic protein or proteins were obtained from the alkaline extract of the nuclear residual proteins by precipitation at pH 6. Judging from the intranuclear localization of the residual acidic proteins and alkali-soluble proteins (40), the two groups of proteins could be the same or similar. Nevertheless, the obvious differences in the amino acid composition between the alkali-soluble and the DOC-treated acidic proteins serve to distinguish them. Whether such deviations
are due to the detergent and alkali treatment cannot be ascertained by the results presented in this paper. One possible factor which may partly contribute to this discrepancy is that the present procedure solubilizes over 90% of the total residual proteins, while dilute alkali extracts about 70% of the total residual proteins (22). The residual acidic proteins must therefore contain some of the alkali-insoluble proteins. The inclusion of the proteins not extracted by alkali probably contributes to the differences in the amino acid composition between the DOC-acidic proteins and the alkali-soluble proteins.

The nature of the complex formation between the acidic proteins and histones is presently unknown. Since the residual proteins are acidic, there could be ionic bonding resulting from interaction between the acidic groups of the residual proteins and the basic residues of the histones, possibly similar to the DNA-histone interaction. If this is the case, the amino acid composition of the acidic proteins would indicate that the interaction is probably not random, but selective. The pH 6 fraction, for example, has the least number of acidic amino acid residues, but its complex-forming activity is the highest. By comparison, the (NH₄)₂SO₄ precipitate fraction which has a high ratio of acidic to basic amino acid residues, exhibits the lowest precipitability. Such an inference would suggest that complex formation between the acidic proteins and the histones may be specific. In light of the current interest regarding histones as the repressors on the DNA template (41, 42), the residual acidic proteins might serve as counter molecules in complex formation between the acidic proteins and the histones. Because of their complex-forming activity with the histones, perhaps functioning in the manner of a de-repressor. Preliminary experiments have shown the DOC-solubilized acidic proteins actively incorporate ¹⁴C-labeled amino acids into protein. The specific role played by the residual acidic proteins in protein synthesis, however, will have to await further information concerning their interplay with DNA and histones. Because of their complex-forming activity with the histones, and also because they contain structures like the "residual chromatin," the acidic proteins may also be an integral part of, or closely related to the chromosome structure.

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REFERENCES