The Amino Acid Composition of Proteins Isolated from the Ribonucleoprotein Particles of Rat Liver*

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There is abundant chemical (1) and morphological (2-4) evidence which suggests that the bulk of the ribonucleic acid of rat liver is concentrated in discrete particles that occur associated with the membranes of the endoplasmic reticulum. Studies both in vivo and in vitro have indicated that labeled amino acids are incorporated into the proteins of these particles at more rapid rates than into other proteins of liver (5, 6). Ultracentrifugal and electrophoretic analyses have revealed the existence of several classes of ribonucleoprotein particles which are interconvertible by means of suitable changes in the ionic environment (7). When these particles are isolated and examined under the same conditions, however, the relative proportions of the several components are found to depend characteristically upon variations in the metabolic state of the animals from which they are obtained (1, 5-10); this suggests the presence of ribonucleoprotein particles of varying degrees of stability (7). Methods have not yet been perfected for obtaining individual, homogeneous components from the nucleic acid and protein moieties which comprise the particles. However, the possibility that the ribonucleoprotein particles participate in steps leading to protein synthesis seemed to justify an investigation of their overall amino acid composition. The present study has revealed that there exists a high proportion of basic amino acids in the protein of the ribonucleoprotein particles.

EXPERIMENTAL

Ribonucleoprotein Particles—Two independent specimens of RNP particles, prepared about a year apart, were studied. Both were obtained from the livers of adult male Wistar rats. Preparation 1 was obtained as previously described (11), except that the particles were washed by three sedimentations from a buffer which was 0.0005 M with respect to KH₂PO₄, K₂HPO₄, and MgCl₂ (7), rather than by sedimentation from a solution containing liver dialysate. Upon ultracentrifugation in 0.1 M NaHCO₃ containing 0.0004 M K₂HPO₄, KH₂PO₄, and MgCl₂, Preparation 1 showed chiefly component B (83 S), with traces of faster and slower components. Electrophoretic patterns of Preparation 2 after dialysis against 0.1 M potassium phosphate and 0.002 M MgCl₂ (pH 8.2) showed 3 per cent of a component with a mobility slower than that of the main component. Since most of the ferritin was removed when the product was precipitated by Ba(OAc)₂ (7), this slow component may represent protein released from the main component during dialysis. Ribonucleic acid was removed from about 90 mg of Preparation 2 by extracting the particles twice with 10 per cent trichloroacetic acid in the cold and four times with 5 per cent trichloroacetic acid at 90°. The resulting precipitate was suspended in 20 volumes of 95 per cent ethanol, which was then adjusted to pH 7 by the careful addition of NaOH. The sediment obtained upon centrifugation was washed twice with 10-m1 portions of acetone, and after drying over night at 60°, yielded 50 mg of a fine powder.

Amino Acid Analyses—After drying at 60 or 100° for 6 hours at 0.1 mm Hg over P₂O₅, 5- to 10-mg aliquots of the proteins isolated from the RNP particles were weighed into tubes suitable for hydrolysis. The proteins were hydrolyzed in 6 N HCl and samples prepared for chromatography by the procedures described by Hirs et al. (12). The hydrolysis was conducted at a temperature of 111° by placing the sealed Pyrex tubes in metal cylinders which were placed in an oil bath submerged in boiling toluene. The amino acid compositions of the 0 N HCl hydrolysates were determined by quantitative photometric ninhydrin analyses (13) of fractions collected after chromatographic analyses performed by the procedures of Moore et al. (14). The resin† that was used for these experiments consisted of the upper one-third of the material that settled under gravity from a 1:1 slurry in 0.2 N sodium citrate, pH 5, after the resin had been coccined through a 325 mesh sieve as prescribed for Dowex 2 J-4953X thermostatic reaction flask, Scientific Glass Apparatus Company, Bloomfield, New Jersey.

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‡ The abbreviation used is: RNP, ribonucleoprotein.

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The basic amino acids were eluted from an 0.9 × 30 cm column of the resin with 0.4 N sodium citrate, pH 5.25, at 50°C. This modification provides a baseline of several fractions between the neutral amino acids and phenylalanine plus tyrosine, as well as between lysine, histidine, and ammonia; baseline follows arginine at about 220 effluent ml.

The tryptophan content of Ba(OH)₂ hydrolysates, prepared in evacuated sealed tubes (16), was estimated after chromatography on 0.9 × 30 cm. columns of potato starch, with 0.1 N HCl as eluent (17). For the estimation of cysteic acid, performic acid oxidation, hydrolysis, and chromatography on Dowex 2 were performed as described by Schram et al. (18).

RESULTS AND DISCUSSION

The amino acid compositions of the proteins of the two preparations of RNP particles are shown in Table I. For the sake of brevity, the compositions calculated from all chromatographic analyses have been combined for each of the preparations. The figures are based on single complete analyses of Preparation 1 after 22 and 70 hours of hydrolysis by 6 N HCl and on single complete analyses of Preparation 2 after 22, 23, and 70 ½ hours of hydrolysis. Unless indicated otherwise, the figures given are the averages of the values found after the different times of hydrolysis; the values agreed in most cases within 3 per cent.

An indicated, however, some of the figures given were calculated by extrapolation to zero time, or were based upon the analysis after hydrolysis for the longer time. Differences between the two preparations may be regarded as significant when they exceed 9 per cent of the values found, which occurred in the case of valine, serine, tyrosine, and ammonia. These differences, which in no case exceeded 15 per cent, most probably were related to the use of two methods of preparation, which might be expected to afford mixtures of slightly different composition. Nevertheless, it is clear that the mixtures from both preparations contain predominantly basic proteins. The figures for total basic residues plus amide ammonia, less total acid residues for Preparations 1 and 2, respectively, arc 7.7 and 9.0 moles per 100 moles of recovered amino acids. For the purpose of comparison, a corresponding figure of 5.6 was calculated for ribonuclease, which may be regarded as a typical basic protein (12).

The compositions of proteins from rat liver RNP particles summarized in Table I are similar to the analyses of RNP particles from rabbit reticulocytes and pea seedlings reported by Ts'o et al. (19). The present results confirm the predictions of these authors that proteins from still other RNP particles might also be found to contain relatively large amounts of basic amino acids.

It is possible that the basicty and the low content of cystine of the proteins of the RNP particles may be significant factors in determining the structural organization of the original nucleoprotein particles, just as these same properties of histones and protamines account for certain structural features of deoxyribonucleoproteins (20). Additional points of similarity between histones and the protein moiety of the RNP particles of rat liver include a low content of tryptophan, a low molecular weight and a tendency to aggregate in solution. It has been possible to isolate the proteins in a soluble form by moving boundary electrophoresis after prolonged dialysis (3 weeks at 5°C) of the RNP particles against phosphate buffer at pH 10.5 and ionic strength 0.2 (21). In dilute solution the isolated proteins had a sedimentation coefficient of 2 S, whereas they underwent extensive aggregation in more concentrated solutions.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>Preparation 1</th>
<th>Preparation 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>5.07</td>
<td>5.57</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>6.40</td>
<td>6.13</td>
</tr>
<tr>
<td>Glycine</td>
<td>5.38</td>
<td>5.33</td>
</tr>
<tr>
<td>Alanine</td>
<td>6.05</td>
<td>5.92</td>
</tr>
<tr>
<td>Valine</td>
<td>4.84</td>
<td>5.51</td>
</tr>
<tr>
<td>Leucine</td>
<td>6.63</td>
<td>6.07</td>
</tr>
<tr>
<td>Cystine</td>
<td>4.00</td>
<td>trace</td>
</tr>
<tr>
<td>Methionine</td>
<td>1.55</td>
<td>1.48</td>
</tr>
<tr>
<td>Proline</td>
<td>3.61</td>
<td>3.41</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>2.76</td>
<td>2.63</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2.35</td>
<td>2.02</td>
</tr>
<tr>
<td>Histidine</td>
<td>4.61</td>
<td>4.93</td>
</tr>
<tr>
<td>Lysine</td>
<td>12.7</td>
<td>13.3</td>
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<tr>
<td>Arginine</td>
<td>19.6</td>
<td>20.8</td>
</tr>
<tr>
<td>Cystine N</td>
<td>3.49</td>
<td>4.85</td>
</tr>
<tr>
<td>Cysteic acid</td>
<td>0.74</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>(100)</td>
<td>(100)</td>
</tr>
</tbody>
</table>

* Calculated from determinations of the amino acids present in 6 N HCl hydrolysates of the proteins after 22 and 70 hours of hydrolysis as described in "Experimental."
* Extrapolated values (cf. (12)).
* The cysteine contents of the 22- and 70-hour hydrolysates agreed within 7 per cent, despite the fact that the original preparation of nucleoprotein particles contained ferritin, the iron of which was expected to facilitate the decomposition of cystine during acid hydrolysis.
* The values for methionine include a 5 per cent correction for losses on the column (14).
* Based on the lysine present in the 70-hour hydrolysate. The value for the 22-hour hydrolysate was lower by 6.4 per cent.

The methods of preparation are described in the text. The figures refer to N as per cent of the total N that was recovered as amino acids and ammonia.

be established whether these similarities are mere coincidence, or whether they reflect structural or metabolic relationships be-
tween the histones of the nucleus and the proteins of the RNP particles.

The present results certainly do not exclude the possibility that typical acidic proteins compose a portion of the RNP particles. In this connection, it is important to emphasize that ribonucleic acid does not always occur associated with basic proteins. Although tropomyosin, the protein moiety of nucleo
tropomyosin, contains a relatively large amount of lysine and arginine, it exhibits a globulin-like solubility and contains far more unamidated aspartic plus glutamic acid than do the proteins from RNP particles discussed above (22). Moreover, the pro-
teins associated with ribonucleic acid in plant viruses, such as tobacco mosaic (23) and turnip yellow mosaic (24), are not predominantly basic.

The possibility that a portion of the protein examined in the experiments presented here becomes associated with ribonucleic acid secondarily during extraction is rendered less likely by the consideration that entities corresponding morphologically to the particles which have been characterized electrophoretically and ultracentrifugally are identifiable in cells before extraction (2–4). Moreover, the fact that RNP particles of microsomal origin have a fairly consistent ratio of protein to ribonucleic acid, regardless of the method of isolation (25, 4, 11, 26), strongly suggests that the protein and nucleic acid are not combined fortuitously. On the other hand, the analyses reported here refer only to proteins that do accompany the RNP particles, and that remain insoluble during the extraction by trichloroacetic acid. In this regard, it may be recalled that some histones are dissociated from deoxyribonucleohistones much more readily than others (27, 28). Under certain conditions, moreover, some histones appear to be soluble in trichloroacetic acid (27).

The present results show that the proteins associated with ribonucleic acid in the RNP particles of rat liver do not have the over-all amino acid composition of “typical” proteins which are synthesized in the liver, such as serum albumin, or of total liver amino acid secondarily during extraction is rendered less likely by the consideration that entities corresponding morphologically to the particles which have been characterized electrophoretically and ultracentrifugally are identifiable in cells before extraction (2–4). Moreover, the fact that RNP particles of microsomal origin have a fairly consistent ratio of protein to ribonucleic acid, regardless of the method of isolation (25, 4, 11, 26), strongly suggests that the protein and nucleic acid are not combined fortuitously. On the other hand, the analyses reported here refer only to proteins that do accompany the RNP particles, and that remain insoluble during the extraction by trichloroacetic acid. In this regard, it may be recalled that some histones are dissociated from deoxyribonucleohistones much more readily than others (27, 28). Under certain conditions, moreover, some histones appear to be soluble in trichloroacetic acid (27).

The present results show that the proteins associated with ribonucleic acid in the RNP particles of rat liver do not have the over-all amino acid composition of “typical” proteins which are synthesized in the liver, such as serum albumin, or of total liver protein (29). Thus, if the rapid incorporation of labeled amino acids into the particles in vivo and in vitro is a reflection of protein synthesis (5, 6), the protein that is synthesized must not remain permanently associated with the particles. The same conclusion, discussed in a recent review by Crick (30), has already been reached from a consideration of the fact that the extent of incorporation of amino acids into the particles reaches a saturation limit rather rapidly (5, 6). Since the bulk of the proteins associated with RNP particles is not chiefly the product of synthesis of the particles, it seems possible that these proteins contribute to the specificity of templates such as have been postulated to control the specific sequence of amino acids in proteins (for a review of this problem see (31)).

5 The hot trichloroacetic acid extract from Preparation 2, contain-
ing the ribonucleic acid, was analyzed for amino acids by Miss Pauline Pecora in Dr. Earl Balis’ laboratory. After hydrolysis with 6 N HCl no ninhydrin positive material could be detected.

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