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Basic and Other Proteins in Microsomes of Rat Liver

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Several physical properties of microsomal ribonucleoprotein particles and the molecular weight of the RNA component of samples from different species have been determined (McQuillen, 1962); the results of these studies suggest that by these criteria ribosomes from all the cells examined appear to be similar in many respects. Some chemical properties of the total protein have been described, e.g. for ribosomes from yeast (Yin & Bock, 1960), pea seedlings (Setterfield, Neelin, Neelin & Bayley, 1960) and *Escherichia coli* (Waller & Harris, 1961; Spahr, 1962), but little is known about the nature of different protein species present. Butler, Cohn & Simson (1960) and Cohn, Simson & Butler (1960) reported that basic proteins were present in ribonucleoprotein preparations obtained from microsomes of rat liver. Extraction of such ribonucleoprotein particles with dilute hydrochloric acid was found to produce a basic fraction in which the proportions of some of the principal *N*-terminal groups were different from those in the total protein. The results of the experiments described below confirm the presence of substantial amounts of basic proteins in pre-

parations of ribonucleoprotein particles obtained by several different methods from microsomes of rat liver.

MATERIALS AND METHODS

All chemicals used were of analytical-reagent grade where available. The preparation of subcellular fractions and the extraction of basic proteins were carried out at 2–4°.

Wistar albino rats were starved for 18–20 hr. before death.

Preparation of subcellular fractions

Microsomes. Rats were killed by breaking their necks; the livers were quickly removed, plunged into ice-cold sucrose and chopped by cutting the tissue with scissors. Samples of liver were homogenized in 0·25M-sucrose (3 ml./g. of tissue) in a Potter–Elvehjem-type homogenizer. While the polythene pestle was being turned at 1700 rev./min. not more than ten up-and-down strokes were carried out. The tissue suspension (11·5 ml. in each tube) was centrifuged (Spinco centrifuge model L, no. 40 rotor) at 15 000$g_{av.}$ for 10 min. A volume (7·0–7·5 ml.) of the supernatant liquid was carefully withdrawn from each tube, the samples from one experiment were pooled and the same volume was pipetted into fresh centrifuge tubes together with sufficient 0·25M-sucrose solution to bring the volume to 11·0 ml. in each tube. Microsome pellets were obtained by centrifuging at 105 000$g_{av.}$ for 45 min.

Lubrol pellet and perfluoro-octanoate pellet. Partial solubilization of the microsome pellet with the non-ionic detergent Lubrol W yielded the Lubrol pellet, which on treatment with sodium perfluoro-octanoate was converted into the perfluoro-octanoate pellet (Cohn & Butler, 1958).

Ammonium sulphate fractions AS_{50} *and* AS_{100}. The Lubrol supernatant liquid was saturated with $MgSO_4,7H_2O$ and the proteins that remained soluble were dialysed against water. After dialysis the clear supernatant liquid was half-saturated with $(NH_4)_2SO_4$. The precipitate (fraction AS_{50}) was collected by centrifuging; complete saturation of the supernatant liquid with $(NH_4)_2SO_4$ produced fraction AS_{100} (Cohn & Butler, 1958). Fraction AS_{50} constitutes 30% and fraction AS_{100} 15% of the protein of the Lubrol supernatant liquid.

Deoxycholate pellet. Method 1. Microsome pellets were suspended by gently homogenizing them in Spinco centrifuge tubes in freshly-made ice-cold 5% (w/v) sodium deoxycholate in 0·03M-tris buffer, pH 8·2 (0·9 ml./pellet). A solution of 0·25M-sucrose and 5 mM-$MgCl_2$ in 0·03M-tris buffer, pH 8·2 (10 ml.), was then added to each tube and ribosomes were separated from solubilized matter by centrifuging at 105 000$g_{av.}$ for 2 hr. The pellets were washed with 0·03M-tris buffer, pH 7·5, containing sucrose (0·25M) and $MgCl_2$ (5 mM) by suspending them by gentle homogenization in Spinco centrifuge tubes and centrifuging at 105 000$g_{av.}$ for 2 hr. The supernatant liquid was then carefully decanted from the ribosome pellet.

Method 2. Unwashed ribosomes were resuspended in 0·03M-tris buffer, pH 7·5, containing sucrose (0·25M) and $MgCl_2$ (5 mM) (10·2 ml./pellet) as described under method 1. Sufficient M-$MgCl_2$ was added to give a final concentration of 50 mM (Takanami, 1960). A precipitate soon appeared which was separated after 30 min. by centrifuging (Spinco model L, no. 40 rotor) at 26 000$g_{av.}$ for 15 min. The pale-grey sediment was taken to be the ribosome pellet.

Method 3. Unwashed ribosomes as prepared by method 1 were resuspended by gently homogenizing them in 0·03M-tris buffer, pH 7·5, containing sucrose (0·15M) and $MgCl_2$ (5 mM). This suspension of ribosomes was carefully pipetted (9·8 ml./pellet) as a layer on top of a volume (22 ml.) of 0·03M-tris buffer, pH 7·5, containing sucrose (0·30M) and $MgCl_2$ (5 mM) previously placed in a tube for the no. 30 rotor of the Spinco model L centrifuge. After the sample had been centrifuged at 78 400$g_{av.}$ for 65 min. the supernatant liquid was carefully decanted from the pale-pink ribosome pellet.

Lubrol–deoxycholate pellet. This was prepared by treating a mitochondrial supernatant liquid with Lubrol W, sodium deoxycholate and potassium chloride, and centrifuging the mixture to obtain ribosomes (Rendi & Hultin, 1960).

Extraction of basic proteins

Ribonucleoprotein pellets, either freshly prepared or, occasionally, after storage at −20°, were suspended by homogenizing them in water to give a total volume of suspension of 2·25 ml. for each pellet, which had been derived from about 3 g. of liver. On the addition of 2N-HCl (0·25 ml.) a cloudy precipitate appeared in the liquid. During the period of extraction the sample was kept moving by gently rocking it. The basic proteins were separated from the acid-insoluble residue by centrifuging at 26 000$g_{av.}$ for 20 min. This extract of basic proteins was freed from HCl and small molecules by dialysis in Visking tubing (20/32 in.) against water on a rocking apparatus for 4–5 hr. Proteins could be recovered from the neutral solution by freeze-drying or by precipitation with acetone at pH 8–9 or by precipitation with trichloroacetic acid. The last-named method yielded the driest and purest product and was used as a routine. It consisted of adding 100% (w/v) trichloroacetic acid to give a final concentration of 15% (w/v). The precipitated proteins were collected by centrifuging, washed twice with acetone and converted into their hydrochlorides by adding 0·1 vol. of 11·6N-HCl to the sample suspended in acetone. The proteins were centrifuged again, washed twice with acetone and dried *in vacuo* at room temperature.

Analysis of subcellular fractions and proteins

Estimations of RNA and total protein of pellets and HCl-insoluble residues and determinations of nitrogen and phosphorus were performed by the techniques described by Cohn & Butler (1958). Analyses of amino acids and of *N*-terminal groups were carried out by a modification of the fluorodinitrobenzene method of Sanger (1945) as described by Phillips (1958) and Phillips & Johns (1959).

RESULTS

In preliminary experiments whole microsome pellets were extracted with dilute hydrochloric acid in an attempt to separate basic proteins. In Table 1 the results of such a fractionation are shown. The hydrochloric acid-soluble proteins have a slightly lower content of aspartic acid and glutamic acid, and a higher content of basic amino

acids, than the total protein of the microsomes. Of the other amino acids determined only phenylalanine and tyrosine showed any differences in their proportions in the two samples of protein. Other differences between the two samples exist in the proportion of N-terminal groups found in them. Total protein of the microsomes is characterized by a high content of proline and glycine and by an appreciable content of serine and of aspartic acid plus glutamic acid. The hydrochloric acid-soluble proteins possess higher proportions of alanine, valine, aspartic acid and glutamic acid, but smaller proportions of proline, glycine and serine N-terminal groups, than the total protein of the microsomes. The Lubrol pellet contains only about one-half the amount of protein but almost all of the RNA of the microsome pellet (Cohn & Butler, 1958), and has a lower content of acidic amino acids than the microsome pellet. Extraction of the Lubrol pellet with 0·2N-hydrochloric acid yields a protein fraction that is slightly more basic than the total protein of the Lubrol pellet. The relations between the proportions of N-terminal alanine, glycine and valine groups in the total protein and in the hydrochloric acid-soluble proteins are similar for the microsome and Lubrol pellets. In the total protein and in the hydrochloric acid-soluble extract of the Lubrol pellet, proline and serine end groups are each present in the same proportions. The hydrochloric acid-soluble proteins contain more aspartic acid and glutamic acid N-terminal groups than does the total protein, but the proportions are less than those found in the corresponding proteins from the microsomes. Further evidence for the heterogeneous nature of the total protein of the microsomes is given by the composition of the proteins of the Lubrol supernatant liquid, which amounts to about one-half of those of the whole microsomes. The proteins of the Lubrol supernatant liquid have a high proportion of aspartic

Table 1. *Amino acid composition of proteins of microsomes and of some microsomal fractions*

Experimental details are given in the text. The amino acids are expressed as moles/100 moles of all amino acids found and no correction has been made for the hydrolytic losses of amino acids. Amide was not determined. The proportions of N-terminal groups are molar percentages of all such groups found.

Subcellular fraction	Microsomes		Lubrol pellet		Lubrol supernatant liquid		
Protein	Total*	HCl-soluble	Total*†	HCl-soluble†	Total	AS_{50}	AS_{100}†
Amino acids							
Aspartic acid (Acidic)	10·1	9·3	9·1	9·1	26·6	9·1	12·5
Glutamic acid (Acidic)	11·5	10·6	10·7	9·5		11·5	11·6
Glycine	6·8	7·9	—	—	7·9	7·0	—
Alanine	7·4	8·7	—	—	7·7	7·7	—
Valine	7·5	6·7	—	—	23·4	7·9	—
Leucine + isoleucine	15·7	13·5	—	—		14·8	—
Serine	6·1	6·6	—	—	5·7	7·0	—
Threonine	5·5	5·2	—	—	6·0	6·0	—
Phenylalanine	5·8	3·9	—	—	5·2	5·6	—
Tyrosine	1·4	2·6	—	—	0·2	2·2	—
Proline	5·9	5·7	—	—	6·5	5·6	—
Histidine (Basic)	2·8	1·6	2·6	2·0	1·3	2·0	1·5
Lysine (Basic)	6·8	10·1	8·0	9·5	3·6	8·5	9·1
Arginine (Basic)	6·8	7·6	6·7	7·1	5·9	5·1	3·8
Basic:acidic ratio	0·76	0·97	0·87	1·00	0·41	0·76	0·60
N-Terminal groups							
Alanine	8	20	10	18	—	6	—
Proline	31	21	29	28	—	15	—
Glycine	25	10	31	15	—	7	—
Serine	10	4	8	8	—	7	—
Aspartic acid	10	5	3	4	—	9	—
Glutamic acid		17	3	6	—	14	—
Valine	3	17	3	13	—	4	—
Leucine + isoleucine	3	—	2	—	—	11	—
Others‡	10	6	11	8	—	27	—
Wt. (g./mole of N-terminal groups)	38 300	85 000§	47 500	77 000§	—	63 000	—

* Butler, Cohn & Simson (1960).

† The values for the basic and acidic amino acids only are given for the sake of clear presentation. Those omitted do not differ appreciably from the results of the analysis of the HCl-soluble proteins of the microsomes.

‡ These include lysine, threonine and tyrosine.

§ Low yield of N-terminal groups; charring of sample during hydrolysis.

acid and glutamic acid residues and are acidic in nature. However, two protein fractions prepared from the Lubrol supernatant liquid, AS_{50} and AS_{100}, possess contents of acidic and basic amino acid residues similar to those in the total protein of the microsomes. The mixture of proteins present in fraction AS_{50} is obviously complex and different from that present in the Lubrol pellet because only three of the N-terminal groups are found in a proportion of more than 10% of the total.

Because of the heterogeneous nature of the total protein of the microsome and Lubrol pellets it was thought desirable to attempt to extract basic proteins from preparations of ribonucleoprotein particles. In Table 2 the amino acid composition of such preparations obtained by five different methods is given. All samples contained approx. 19% of acidic amino acids. The ratios of basic to acidic amino acids are similar in the protein of the perfluoro-octanoate and the deoxycholate pellets prepared by any of the techniques described above. Only the protein of the Lubrol–deoxycholate pellet has a ratio of basic to acidic amino acids that is well above unity. For comparison, the analysis by Crampton & Petermann (1959) of a preparation of microsomal ribonucleoprotein that was extensively purified by repeated reprecipitation and dialysis is shown.

Table 3 shows the proportion of protein extractable from different ribonucleoprotein preparations during various periods of time. Extraction for 0·5 hr. produces as much or even slightly more protein than extraction for 15·5–16·5 hr. A small quantity of additional protein could be extracted when the residue remaining after an extraction lasting for 0·5 hr. was re-extracted for 15·5–16·5 hr. Differences exist in the amount of protein extracted/mg. of RNA. On this basis the perfluoro-octanoate pellet yields more protein than any of the other ribonucleoprotein preparations, indicating that proteins which are present in the perfluoro-octanoate pellet but not in the deoxycholate and Lubrol–deoxycholate pellets are extracted by 0·2N-hydrochloric acid.

The ultraviolet-absorption spectra of two extracts obtained by successive treatments of deoxycholate pellets (method 1) with 0·2N-hydrochloric acid for 0·5 and 15·25 hr. are illustrated in Fig. 1. Although the curves are not identical, they are both characteristic of protein and have maxima at 275 mμ and minima at about 250 mμ. The second extract has a higher specific extinction coefficient than the first extract, but the $E_{275}:E_{260}$ ratio is lower for the second (1·13) than for the first (1·51). Protein mixtures prepared by one extraction for 15–16 hr. have ratios of intermediate values. Undialysed extracts have a somewhat higher extinction, particularly at 260 mμ, which may be due

Table 2. *Amino acid composition of protein of microsomal ribonucleoprotein*

Experimental details are given in the text. The amino acids are expressed as moles/100 moles of all amino acids found and no correction has been made for the hydrolytic losses of amino acids. Each value represents the mean of the results from the stated number of preparations. Where no values are given they were found not to differ appreciably from those in the first column. Amide was not determined.

Ribonucleoprotein preparation		Deoxycholate pellet (method 1)	Deoxycholate pellet (method 2)	Deoxycholate pellet (method 3)	Lubrol–deoxycholate pellet	Perfluoro-octanoate* pellet	Deoxycholate pellet (sample 1)†	Deoxycholate pellet (sample 2)†
No. of preparations		2	1	1	3	2	1	1
Aspartic acid	Acidic	19·2	19·2	9·1	18·5	8·1	8·58	8·24
Glutamic acid	Acidic			10·4		10·9	9·28	9·05
Glycine		8·0	—	—	—	—	—	—
Alanine		8·3	—	—	—	—	—	—
Valine		8·3	—	—	—	—	—	—
Leucine + isoleucine		14·6	—	—	—	—	—	—
Serine		5·6	—	—	—	—	—	—
Threonine		5·2	—	—	—	—	—	—
Phenylalanine		4·0	—	—	—	—	—	—
Tyrosine		2·8	—	—	—	—	—	—
Proline		5·0	—	—	—	—	—	—
Histidine	Basic	2·2	1·7	2·0	2·3	2·4	2·21	2·42
Lysine	Basic	10·0	10·3	10·0	11·3	8·2	9·09	9·79
Arginine	Basic	6·8	6·0	5·7	8·2	7·9	7·03	7·67
Basic:acidic ratio		0·99	0·94	0·91	1·18	0·97	1·03	1·15

* Butler, Cohn & Simson (1960). † Crampton & Petermann (1959).

Table 3. *Proportion of protein extractable with* 0·2 N-*hydrochloric acid from ribonucleoprotein pellets*

Experimental details are given in the text. Each value represents the mean of the results from the stated number of preparations.

Ribonucleoprotein: Preparation	Ribonucleoprotein: Protein (%)*	Period of extraction with 0·2 N-HCl (hr.)	Amount of protein extracted (%)	mg. of protein extracted / mg. of RNA	No. of preparations
Perfluoro-octanoate pellet	69	16·5	29	0·64	1
	72	0·5	27	0·63	
		then 15·25	2	0·05	1
Deoxycholate pellet (method 1)	57	0·5	32	0·42	
		then 15·25	5	0·05	2
Deoxycholate pellet (method 2)	54	15·5	26	0·30	1
Deoxycholate pellet (method 3)	58	15·5	21	0·29	1
Lubrol–deoxycholate pellet	54	6	25	0·20	1
	51	0·5	39	0·36	
		then 15·25	5	0·05	3
	51	15·5	34	0·29	2

* The presence of phospholipid was ignored when calculating the percentage composition.

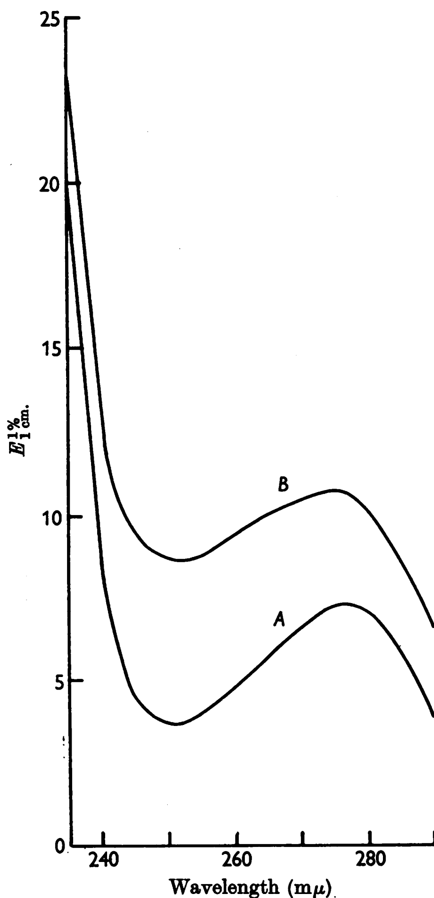

Fig. 1. Ultraviolet-absorption spectra of dialysed extracts of basic proteins from deoxycholate pellet (method 1). Experimental details are given in the text. *A*, Extraction for 30 min.; *B*, subsequent extraction of residue for 15·25 hr.

to a small quantity of breakdown products of RNA. Approx. 90% of the RNA of the ribonucleoproteins can be recovered in the hydrochloric acid-insoluble residue.

Table 4 shows the proportions of basic and acidic amino acids in basic and hydrochloric acid-insoluble proteins from various ribonucleoprotein preparations. The basic proteins from all sources are much richer in basic amino acids but have a slightly lower content of acidic amino acids than the hydrochloric acid-insoluble proteins. The remaining amino acids are found in proportions similar to those given for whole ribonucleoprotein pellets (Table 2). The basic proteins are distinct from the hydrochloric acid-insoluble proteins in the abundance of two of the principal *N*-terminal groups: little glycine but much alanine is present in the basic proteins whereas the hydrochloric acid-insoluble proteins are poor in alanine but rich in glycine.

DISCUSSION

The results of extracting various ribonucleoprotein preparations from rat liver with dilute hydrochloric acid show that under the experimental conditions used a fractionation into a group of basic and one of acidic proteins has occurred. A portion of about 40% of the total protein of ribosomes, which are represented in this investigation by the deoxycholate and Lubrol–deoxycholate pellets, is basic in character and markedly differs from the residual hydrochloric acid-insoluble proteins in the pattern of the principal *N*-terminal groups present. Analysis of the proteins extractable from microsomes with dilute hydrochloric acid does not clearly establish the presence of these basic proteins because not only basic but also some of the acidic proteins in microsomes are soluble in dilute

Table 4. *Proportion of basic and acidic amino acids in basic and hydrochloric acid-insoluble proteins from microsomal ribonucleoprotein*

Experimental details are given in the text. The amino acids are expressed as moles/100 moles of all amino acids found and no correction has been made for the hydrolytic losses of amino acids. Each sample analysed was obtained from a separate preparation of the specified protein fraction unless otherwise stated.

The values represent the means of the stated number of samples analysed.

Ribonucleoprotein preparation ...	Perfluoro-octanoate pellet		Deoxycholate pellet (method 1)			Deoxycholate pellet (method 2)	Deoxycholate pellet (method 3)	Lubrol–deoxycholate pellet		
Period of extraction (hr.)	17		0·5	then 15·25		15·5	15·5	0·5	then 15·25	
Protein fraction	Basic	HCl-insoluble	Basic	Basic	HCl-insoluble	Basic	Basic	Basic	Basic	HCl-insoluble
No. of samples analysed	4*	2	1†	1†	1†	1	1	1‡	1‡	1‡
Amino acids										
Aspartic acid + Glutamic acid (Acidic)	18·2	9·4, 10·2	7·4, 11·0	17·6	20·0	17·4	12·1, 5·5	18·3	5·2, 13·1	
Aspartic acid										9·1
Glutamic acid										12·2
Histidine (Basic)	2·1	1·8	1·9	2·1	1·8	2·3	1·9	1·4	2·0	1·8
Lysine (Basic)	12·4	8·1	14·3	13·6	8·0	14·2	13·9	12·6	11·8	9·2
Arginine (Basic)	8·1	5·5	8·0	7·8	7·6	6·4	6·4	8·9	9·1	7·1
Basic : acidic ratio	1·24	0·79	1·32	1·34	0·87	1·32	1·26	1·25	1·25	0·85
N-Terminal amino acids										
Alanine	27	12	28	—	9	—	—	25	—	6
Proline	24	22	22	—	19	—	—	22	—	23
Glycine	13	33	16	—	31	—	—	15	—	41
Serine	9	12	15	—	16	—	—	18	—	12
Others§	27	21	19	—	26	—	—	20	—	18
Wt. (g./mole of *N*-terminal groups)	74 000‖	56 000	36 000	—	51 000	—	—	40 000	—	62 000

* Analysis of *N*-terminal groups was carried out on one of the samples only.
† Corresponding protein fractions from two preparations were pooled before analysis.
‡ Corresponding protein fractions from three preparations were pooled before analysis.
§ These include aspartic acid, glutamic acid, leucine + isoleucine, and lysine.
‖ Low yield of *N*-terminal groups; charring of sample during hydrolysis.

hydrochloric acid. Only a preparation of ribonucleoprotein particles, which by themselves contain a higher proportion of basic proteins than whole microsomes, yields an extract rich in basic proteins.

The average weight per *N*-terminal group in these basic proteins is 36 000–40 000. This value is lower than that for the hydrochloric acid-insoluble proteins which, being insoluble, give a decreased yield of *N*-terminal amino acid residues by the fluorodinitrobenzene method. Curry & Hersh (1962) have reported a value of 24 400, determined by the sedimentation-equilibrium method, for the average molecular weight of the total protein of ribosomes from calf liver. The reason for the difference between these two values might be explained either by the tendency of basic proteins to aggregate or by the presence of a proteolytic enzyme that has been reported in ribosomes of *E. coli* and that could affect the composition of ribosomal proteins during the prolonged period of extraction at pH 7·6 employed by Curry & Hersh (1962). It is noteworthy that protein prepared from ribosomes of *E. coli* by extraction with 67 % (v/v) acetic acid has been found to have an average molecular weight of 25 400 (Waller & Harris, 1961).

The overall amino acid composition of the protein of ribosomes from a number of different sources appears to be similar (Ts'o, Bonner & Dintzis, 1958), but the polypeptide chains of these proteins possess different *N*-terminal groups: thus in the protein from *E. coli* they are alanine and methionine (Waller & Harris, 1961), and in the protein from the buds of pea seedlings alanine and valine account for over 50 % of such end groups (Setterfield *et al.* 1960). However, in ribosomes from rabbit reticulocytes *N*-terminal alanine, proline, glycine and serine groups have been found in the basic and hydrochloric acid-insoluble proteins in similar proportions to those found in corresponding fractions from ribosomes of rat liver (Cohn, 1962).

SUMMARY

1. Microsomes and preparations of ribonucleoprotein particles were extracted with 0·2 N-hydrochloric acid and the amino acid composition of the proteins was determined. A portion of about 40 % of the total protein could be extracted from purified ribosomes.

2. The extracted proteins possessed 24 % of basic and 18 % of acidic amino acid residues.

3. The principal *N*-terminal groups found were alanine, proline, glycine and serine. There were marked differences in the proportions of alanine and glycine contained in the basic and the hydrochloric acid-insoluble proteins.

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