Oleinick, N. L., and Corcoran, J. W. (1967), *Federation Proc. 26*, 285.

Tanaka, K., Teraoka, H., Nagira, T., and Tamaki, M. (1966), *Biochim. Biophys. Acta 123*, 435.

Taubman, S. B., and Corcoran, J. W. (1965), 5th Interscience Conference on Antimicrobial Agents and Chemotherapy, 4th International Congress of Chemotherapy, Washington, D. C., 1963, p 86.

Taubman, S. B., Jones, N. R., Young, F. E., and Corcoran, J. W. (1966), *Biochim. Biophys. Acta 123*, 438.

Taubman, S. B., So, A. G., Young, F. E., Davie, E. W., and Corcoran, J. W. (1964), *in* Antimicrobial Agents and Chemotherapy, Ann Arbor, Mich., 1963, p 395.

Taubman, S. B., Young, F. E., and Corcoran, J. W. (1963), *Proc. Natl. Acad. Sci. U. S. 50*, 955.

Vazquez, D. (1966), *Biochim. Biophys. Acta 114*, 277.

Wolfe, A. D., and Hahn, F. E. (1964), *Science 143*, 1445.

Zubay, G. (1962), *J. Mol. Biol. 4*, 347.

Characterization of Some of the Proteins of the Large Subunit of Rat Liver Ribosomes*

Mary G. Hamilton and Mary E. Ruth

ABSTRACT: The four chief electrophoretic components of the proteins of the large subunit of rat liver ribosomes have been separated and their molecular weights measured. Two of these components were obtained as electrophoretically homogeneous fractions with molecular weights of 14,800 and 28,600. For the other two, molecular weights were obtained by extrapolation of measurements on mixtures but the values, 16,000 and 19,000, probably are fair estimates of the true molecular weights. The similar sizes of electrophoretically distinguishable components suggest that the ribosomal proteins differ in composition as well as size.

The electrophoretic heterogeneity of ribosomal proteins in starch and polyacrylamide gels is well known (Waller and Harris, 1961; Leboy *et al.*, 1964; Hamilton and Ruth, 1966; MacQuillan and Bayley, 1966; Low and Wool, 1966). Because of the sieving properties of the gels, migration is affected by molecular size and shape as well as by net charge and it is difficult to interpret the patterns. Thus: (1) the components might be all the same size, but differ in composition; (2) they might be alike in composition, but form complexes of various sizes; (3) they might vary in both size and composition; and (4) as an added complication, they may exhibit conformational isomerism. To determine which of these descriptions applies to the population of ribosomal proteins, the mixture has been fractionated electrophoretically on a polyacrylamide column and the molecular weights of the fractions have been measured by equilibrium centrifugation. Because the electrophoretic distributions of the proteins from the two ribosomal subunits differ, the subunits were first separated and the more stable large subunit of rat liver ribosomes has been used as a source of protein. Two of the fractions were monodisperse both in the ultracentrifuge and on reexamination by electrophoresis. These had molecular weights of 14,800 and 28,600. The other fractions were not monodisperse by electrophoresis although some appeared to be monodisperse in the ultracentrifuge.

Experimental Procedures

Isolation of Ribosomes and Separation of Subunits.[1] Ribosomes were isolated from the livers of fasted, adult male rats and purified by the procedures of Petermann and Pavlovec (1963) and Schwartz and Petermann (1966) which involve the use of sodium deoxycholate, bentonite, a pH 8 wash, and $MgCl_2$ precipitation. This method yields electrophoretically pure ribosomes. Ribosomes were dissociated to subunits by passage through a Sephadex G-100 column in 0.1 M KCl–0.001 M potassium phosphate (pH 7.0) (Petermann and Pavlovec, 1966), dialyzed overnight against

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[1] All procedures were carried out in a cold room at temperatures from 5 to 10°. Stock solutions of urea 10 (M) were treated with charcoal before use.

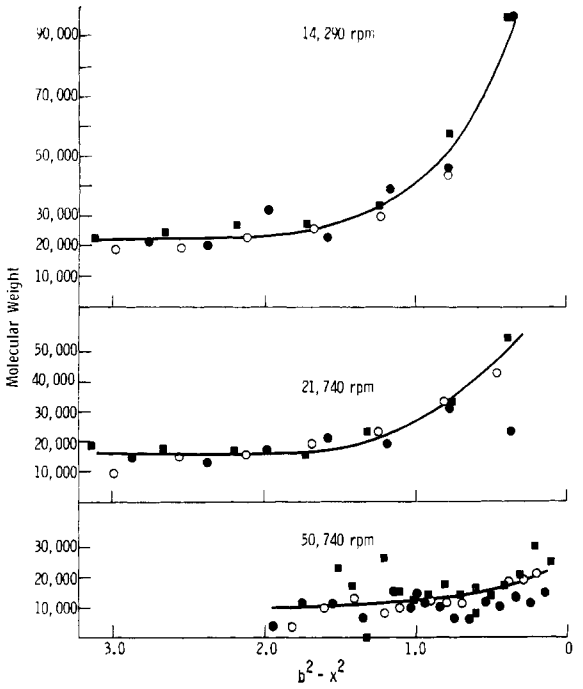

FIGURE 1: Equilibrium centrifugation of the RCM proteins of dissociated ribosomes. Plots of the data from runs at three speeds at initial concentrations of 1.05 (●), 0.65 (○), and 0.35 mg/ml (■). The point-average molecular weight is plotted against x^2. (b is the position of the base of the solution.)

the same buffer with KCl reduced to 0.03 M, and shell frozen in the presence of 3% sucrose. The subunits were separated by centrifugation for 18 hr at 20,000 rpm in a 10–25% sucrose gradient in a B-XV zonal rotor (Anderson, 1966). In addition to the expected subunits a slowly sedimenting peak was always present. This material accounted for less than 10% of the total, was polydisperse, and contained RNA of about 5 S and some protein. The subunits were pelleted from the pooled peak fractions and resuspended in small volumes of 0.03 M KCl–0.001 M potassium phosphate (pH 7).

The RNA and protein content of the large subunit were determined by the methods of Petermann and Pavlovec (1966). It was found to be depleted in protein relative to the intact ribosomes with 45% instead of 50% protein.

Extraction of Ribosomal Protein. The LiCl method of Curry and Hersh (1962), which extracts over 95% of the protein from mammalian ribosomes, was used. To obtain RNA-free protein, however, it was necessary to dissociate the ribosomes to subunits before extraction, since the 4S RNA bound to intact ribosomes is soluble in 2 M LiCl (Barlow *et al.*, 1963). The solution of dissociated ribosomes or isolated subunits (7–8 mg/ml) was made 2 M in LiCl and allowed to stand at 5° for 2 days. The RNA precipitate and any undissociated ribonucleoprotein were removed by centrifugation at 105,000*g* for 1 hr. The protein solution was first dialyzed against 6 M urea–0.03 M sodium acetate[1] (pH 5.6) to remove the LiCl, and then concentrated by dialysis against dry Sephadex G-200.

Chemical Modification of Ribosomal Protein. The ribosomal proteins were reduced with mercaptoethanol and carboxymethylated with iodoacetate under the conditions set by Crestfield *et al.* (1963).

Analytical Polyacrylamide Gel Electrophoresis. The pH 4.6, two-gel, discontinuous buffer (acetate–β-alanine) system of Reisfeld *et al.* (1962) was used in small columns (5-mm i.d.). The lower gel was 7.5% polyacrylamide. All the solutions contained 6 M urea. A current of 3 ma/column was applied for 90 min. The amido black stained gels were photographed for densitometry as described by Burns and Pollak (1963).

Preparative Electrophoresis in a Polyacrylamide Column. The apparatus of Duesberg and Rueckert (1965) was used with their gel system II (4% polyacrylamide) (pH 4.6) but in 6 M urea. An overnight preliminary run of the lower gel in its buffer was made to eliminate excess catalyst and unidentified ultraviolet-absorbing substances eluted from the gel. About 6 mg of protein was applied in a slurry of Sephadex G-200 and 8 M urea over the spacer gel. The conditions of electrophoresis were the same as those described by Duesberg and Rueckert (1965). Samples (1 ml) were collected, dialyzed against 6 M urea–0.03 M sodium acetate (pH 5.6), and concentrated by dialysis against dry Sephadex G-200 to about 0.2 ml. Part of each sample was subjected to analytical electrophoresis. The remainder was redialyzed against 4 M urea–0.2 M LiCl–0.03 M sodium acetate (pH 5.6) for equilibrium centrifugation.

The dialysis membrane used was Visking no. 18 tubing. Before use it was boiled for 15 min first in 0.1% sodium dodecyl sulfate–0.1% Na_2EDTA and then in distilled water. The dialysis chambers were made from 2-ml vials by boring out the plastic cover to hold a piece of membrane. The samples were mixed at intervals by inverting the vials. The outside solution was stirred.

Molecular Weight Measurements. Short-column (2–3 mm) equilibrium centrifugation of dialyzed samples was carried out in the multicompartment cell of Yphantis (1964). The ultracentrifuge had an ultraviolet absorption optical system, a monochromator, and a photoelectric scanner. Samples were run at temperatures below 10° at 21,740 rpm for 24 hr. In a few cases, the centrifuge was run at 14,290 rpm until equilibrium was reached, about 24 hr; then the centrifuge was accelerated to 21,740 rpm and a second condition of equilibrium attained. Solutions were scanned at 275 or 280 mμ. The scanner gives tracings from which one can compute the net absorbance of the solution by reference to a calibration curve (Schachman and Edelstein, 1966). Molecular weights were calculated from the slopes of plots of the logarithm of absorbance against x^2. A partial specific volume of 0.739, calculated from the amino acid composition of the total protein (Cramp-

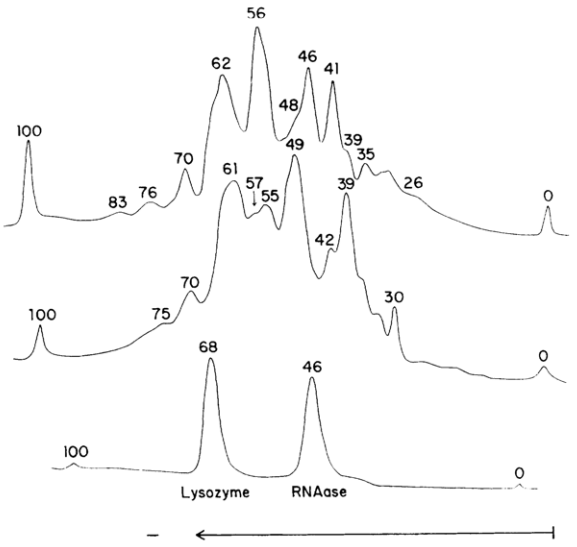

FIGURE 2: Densitometer traces of disc electrophoretic patterns. Top: The proteins of dissociated rat liver ribosomes; middle: RCM ribosomal proteins; bottom: mixture of egg white lysozyme and pancreatic ribonuclease. Standard conditions of electrophoresis: pH 4.6, 6 M urea. The numbers indicate mobilities expressed as percentages of the distance migrated from the origin at the right to the cationic front.

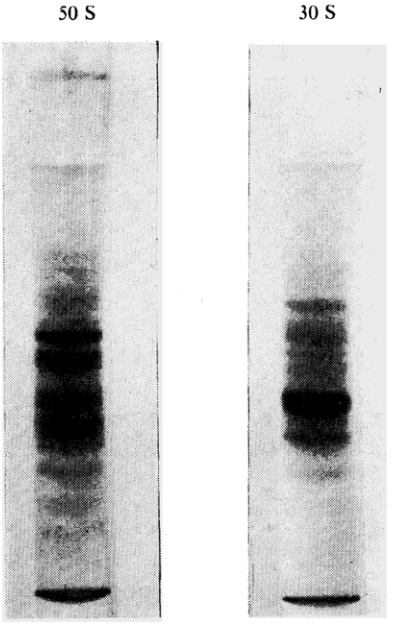

FIGURE 3: Comparison of the proteins from the separated large and small subunits of ribosomes by disc electrophoresis. Migration is from the origin at the top to the cathode at the bottom.

ton and Petermann, 1959), was used. For the mixture of *E. coli* ribosomal proteins, Möller and Chrambach (1967) measured $\bar{v}$ in urea solutions and found it to be close to that calculated from the amino acid composition. This suggests that preferential interactions with urea do not occur to a significant extent. The density of the solvent was measured at 8° in a 3-ml pycnometer. To test the technique the molecular weight of a well-characterized small protein, egg white lysozyme (Pentex, three-times recrystallized), was checked in the urea–LiCl–acetate buffer.

To determine the resolving power of the method for proteins with molecular weights ranging from 14,000 to 18,000, mixtures containing varying proportions (1:4, 1:1, and 4:1) of lysozyme, molecular weight of 14,600, and sperm whale myoglobin (gift of Dr. E. Breslow), 17,800, in 0.1 M sodium phosphate (pH 7) were studied by equilibrium centrifugation at 20,410 and 29,500 rpm. No curvature was detected in the plots of log c *vs.* x^2 and the experimentally determined weight-average molecular weights agreed with the values calculated for the mixtures within 10%.

Results

Characterization of Reduced, Carboxymethylated Ribosomal Proteins. Preliminary experiments were carried out on the unfractionated protein from dissociated ribosomes. A sedimentation velocity run with schlieren optics in a synthetic boundary cell at a concentration of 2 mg/ml showed a single boundary that spread very quickly with time, decreasing in area by 15% within 1 hr of centrifugation at 21,740 rpm. Figure 1 shows data obtained with ultraviolet absorption optics from equilibrium centrifugation at three lower concentrations and three speeds. The molecular weight is plotted as a function of position in the solution as suggested by Yphantis (1964). By his criteria there is no concentration dependence, since the points at the three concentrations superimpose, but the solute is not homogeneous in size, since the lines are curved. Thus reduction and carboxymethylation did not convert the population of molecules to a homogeneous size class. Further evidence for dispersity is seen in the different minimum molecular weights obtained at different speeds: 22,000 at 14,290 rpm; 16,000 at 21,740 rpm; and 10,000 at 50,740 rpm.

The effect of reduction and carboxymethylation on the electrophoretic behavior of the proteins is shown in Figure 2. As expected, the addition of new charged groups results in some changes in the band pattern.

Also shown in Figure 2, to illustrate the resolving power of the gel system, is the pattern of a mixture of two pure proteins of similar molecular weights but different chemical compositions, lysozyme and bovine pancreatic ribonuclease. In contrast, polylysine, a population of molecules of identical chemical composition but different sizes, examined under the same conditions, gave no discrete bands but a smear throughout the central region of the gel.

Electrophoretic Comparison of the Proteins of the 50S and 30S Ribosomal Subunits. Figure 3 shows the

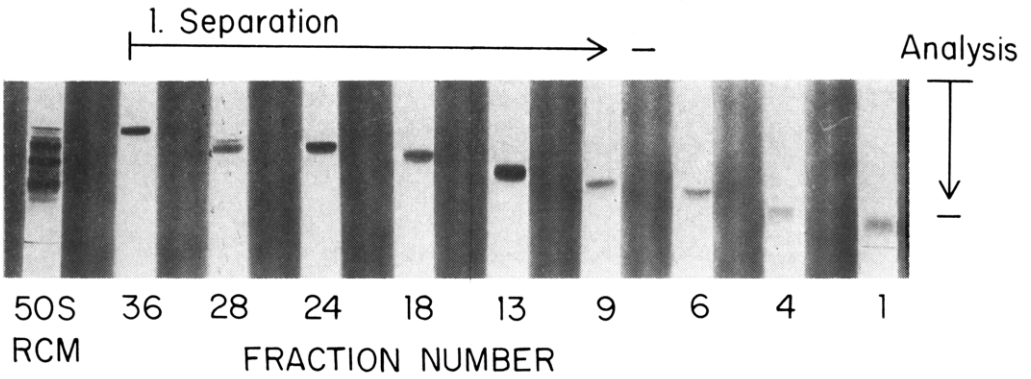

FIGURE 4: Electrophoretic analyses of some of the fractions of RCM proteins obtained by preparative electrophoresis. The pattern of the starting material is at the left. The numbered samples are arranged in order of their elution from right to left.

patterns obtained on the proteins of the large and small subunits. Both qualitative and quantitative differences can be seen; at least 17 bands can be counted in the 50S profile and 12 in the 30S. The most obvious difference is a prominent band (or bands), representing 40% of the material, in the central region of the 30S pattern. In general, the proportion of faster (*i.e.*, more basic or smaller) components is slightly greater in the 30S than in the 50S protein pattern.

Electrophoretic Fractionation of RCM[2] *Proteins of the Large Subunit.* Thirty-six 1-ml fractions were collected from the preparative electrophoresis column. Each was examined for identification and monodispersity by electrophoresis in a small analytical gel; some of these gels are shown in Figure 4. Only two of the components were separated in fractions that were monodisperse on reanalysis, and each was recovered in three or four fractions. The other components were recovered as mixtures of two and sometimes three components, again spread over three or four fractions.

Equilibrium Centrifugation of Isolated Fractions. The results of the molecular weight measurements are given in Table I, which also lists the components and their relative amounts, calculated from the areas under the peaks of Figure 5, the electrophoretic pattern of the starting material. The electrophoretically monodisperse fractions containing the 64 and 40% components were also monodisperse on ultracentrifugation, with molecular weights of 14,800 and 28,600, respectively. Although most of the other fractions were not electrophoretically homogeneous, they appeared to be monodisperse in the ultracentrifuge. For these samples molecular weights were estimated by extrapolation of the data as shown in Figure 6, where the weight-average molecular weight is plotted against electrophoretic composition as determined by planimetry of the gel patterns. Graph 1 of Figure 6 shows mixtures of the 69 and 64% components; a value of 11,300 for the 69% component is obtained by extrapolation. Graph 2 shows mixtures of the 54 and 50% components; their molecular weights by extrapolation are 16,300 and 19,400. Graph 3 gives data obtained on fractions containing the 50 and 46% components, which behaved quite differently from the others in the ultracentrifuge. The plots of log c *vs.* x^2 were straight lines but higher molecular weights were obtained at lower speeds; in the most extreme example the apparent molecular weights were 32,000 at the lower

TABLE I: The Molecular Weights and Approximate Numerical Distribution of the Proteins of the Large Subunit of Rat Liver Ribosomes.

Component[a] (% distance migrated)	Distribn from Disc Electrophoresis[a] (%)	Mol Wt	No. of Molecules Assuming Total Wt of 1.3 × 10^6
73–86	8	10,000[e]	10
69	4	11,300[f]	5
64[b]	20	14,800[g] ± 13%	18
60	2	(15,000)[i]	2
54	12	16,300[f]	10
50[c]	20	19,400[f]	13
46	2	11,000[h]	2
40[d]	20	28,600[g] ± 12%	9
16–35	12	(15,000)[i]	10
			Sum 79

[a] See Figure 5. [b] Isolated protein migrated at 60% position (see Discussion). [c] Isolated protein migrated at 45% position. [d] Isolated protein migrated at 36% position. [e] Single measurements on mixtures of three or four components. [f] Extrapolated values (see Figure 6). [g] Electrophoretically monodisperse; average of measurements on three samples. [h] See graph 3 of Figure 6. [i] Assumed value.

[2] Abbreviation used: RCM, reduced, carboxymethylated.

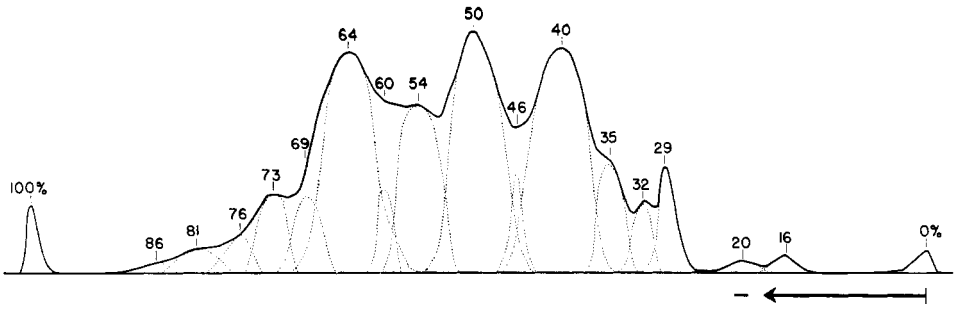

FIGURE 5: Detailed analysis of the electrophoretic pattern of RCM proteins of the large subunit. See legend of Figure 2 for a definition of the numerical labels. Visual inspection of the gel was used as a guide in assigning areas to the components. For example, there were small, sharp bands at the 60 and 46% positions which are not clearly defined in the densitometer trace.

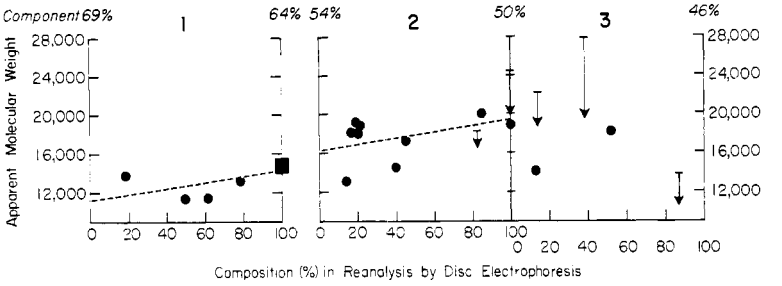

FIGURE 6: Molecular weights of protein mixtures obtained by electrophoretic fractionation of the RCM proteins of the large subunit. The plots of log c *vs.* x^2 were straight lines. The weight-average molecular weight for each fraction is plotted against its electrophoretic composition. The arrows indicate experiments where centrifugation was carried out first at 14,290 rpm (the tail of the arrow) and then at 21,740 rpm (arrow head). (■) Average of measurements on three samples of the 64% component. In graphs 1 and 2 the line is the least-squares fit of the points.

speed and 24,000 at the higher speed. Moreover, for a few samples from this region molecular weights could not be calculated because of curvature in the log plots. The 46% component seems to interact strongly with the 50% component. Its molecular weights can be taken as approximately 11,000. A few measurements on fractions containing the minor fast components, 86% to 73%, gave molecular weights close to 10,000.

Discussion

The heterogeneity of the proteins of *E. coli* ribosomes has been investigated by Waller (1964), Duerre (1964), and Möller and Chrambach (1967), among others. The last authors examined the proteins by various physical techniques and obtained an average molecular weight of 26,000. In equilibrium centrifugation some polydispersity was noted; M_w was 23,000 and M_z was 27,000.

In contrast we found the unfractionated proteins of rat liver ribosomes to be considerably more polydisperse than the *E. coli* proteins. In equilibrium runs, not presented here in detail, for the unmodified proteins in 6 M guanidine hydrochloride–0.5 M sodium acetate (pH 5), M_w was 26,000 and M_z was 33,000. Moreover, we have observed interactions among the proteins in moving-boundary electrophoresis in the neutral pH range and in two-dimensional gel electrophoresis at various pH values (M. G. Hamilton and M. E. Ruth, in preparation). Because of these indications of heterogeneity, we have not carried out further physical studies of the protein mixture. Rather, since our aim is to characterize the protein population as to size and number of molecules, we have begun to fractionate the mixture by preparative electrophoresis. Some of the proteins of the large subunit have been partially characterized and we submit these results as a preliminary survey of the protein complement of the large subunit of rat liver ribosomes.

In the present experiments we have isolated only two of the 17 components of the large subunit, probably because they are present in relatively large amounts. The other two main components were obtained as mixtures, in varying proportions, with their neighboring proteins, and we have estimated their molecular weights by extrapolation instead of by averaging the values. These four, the most abundant species representing 72% of the total protein, in order of their electrophoretic mobilities, have molecular weights of about 15,000, 16,000, 19,000, and 29,000. While the 29,000 component might be a dimer of

one of the others, the presence of urea militates against that possibility. Further, conformational isomerism among components of similar sizes perhaps can be ruled out, since RCM proteins probably are random coils in 6 M urea. Thus, the different mobilities of components with approximately the same molecular weights suggest that the molecules differ chemically; we hope to confirm this by amino acid analyses[3] and peptide mapping.

In the experiments reported here the amount of protein recovered in each fraction was about 100 μg, insufficient to permit the investigation of electrophoretic behavior at several pH values that is essential to establish the purity of a protein. Electrophoretic analysis under the standard conditions was used to identify the components in the isolated fractions with the components in the original mixture. It might be assumed that the components would migrate at the same positions after isolation as they did in the mixture. However, from consideration of the relative abundance of the components isolated, we found that assumption to be only an approximation. Thus the component that migrated at the 60% position after isolation corresponds in quantity recovered to the 64% component of the mixture; the 46% isolated component corresponds to the 50%; and the 36% to the 40% component of the mixture.

For the present we are assuming that the electrophoretic pattern of the original mixture represents the true distribution of components, that is, that there is no differential uptake of the amido black stain. Recently, Traut (1966) has compared the electrophoretic distribution of radioactively labeled *E. coli* ribosomal proteins with that of the amido black stained pattern. He found that the stain underestimates the more slowly migrating components. Although the same situation may apply to rat liver ribosomal proteins, the protein patterns of rat liver and *E. coli* ribosomes do differ greatly, as we, in unpublished experiments, and others (Low and Wool, 1966) have shown.

Despite these difficulties we can roughly estimate the number of molecules of various sizes in the subunit. The 50S subunit of rat liver ribosomes has a molecular weight of 2.8 million as determined by equilibrium centrifugation at high speeds (Yphantis, 1964) and low concentrations, with ultraviolet absorption optics (M. G. Hamilton, in preparation). Since the protein content is 45%, the amount of protein in the subunit is about 1.3×10^6 daltons. The numerical distribution of the components calculated from these data is given in the last column of Table I. Thus, over one-half of the molecules (41) have molecular weights between 15,000 and 19,000, nine have a molecular weight of 29,000, and the remaining 29 probably have molecular weights between 10,000 and 15,000. The latter group includes those for which we have little or no data at present.

[3] Recently Dr. Josephine Salser of this Institute has found marked differences in amino acid composition between the 64 and 40% components; the mole fractions of basic, acidic, and neutral amino acids are 0.335, 0.154, and 0.511 for the 64% component, and 0.221, 0.186, and 0.593 for the 40% component.

In conclusion, these results, showing that individual ribosomal proteins differ in size, support the growing evidence (Waller, 1964; Leboy *et al.*, 1964; Warner, 1966; Möller and Chrambach, 1967) for great complexity in the structure of ribosomes. Of great interest are the recent demonstrations of functional specificities in the protein population of *E. coli* ribosomes (Traub *et al.*, 1966; Staehelin and Meselson, 1966).

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References

Anderson, N. G. (1966), *Science 154*, 103.

Barlow, J. J., Mathias, A. P., Williamson, R., and Gammack, D. B. (1963), *Biochem. Biophys. Res. Commun. 13*, 61.

Burns, D. A., and Pollak, D. J. (1963), *J. Chromatog. 11*, 559.

Crampton, C. F., and Petermann, M. L. (1959), *J. Biol. Chem. 234*, 2642.

Crestfield, A. M., Moore, S., and Stein, W. H. (1963), *J. Biol. Chem. 238*, 622.

Curry, J. B., and Hersh, R. T. (1962), *Biochem. Biophys. Res. Commun. 6*, 415.

Duerre, J. A. (1964), *Biochim. Biophys. Acta 86*, 490.

Duesberg, P. H., and Rueckert, R. R. (1965), *Anal. Biochem. 11*, 342.

Hamilton, M. G., and Ruth, M. E. (1966), Abstracts, 10th Annual Meeting of the Biophysical Society, Feb 1966, Boston, Mass.

Leboy, P. S., Cox, E. C., and Flaks, J. G. (1964), *Proc. Natl. Acad. Sci. U. S. 52*, 1367.

Low, R. B., and Wool, I. G. (1966), *Science 155*, 330.

MacQuillan, A. M., and Bayley, S. T. (1966), *Can. J. Biochem. 44*, 1221.

Möller, W., and Chrambach, A. (1967), *J. Mol. Biol. 23*, 377.

Petermann, M. L., and Pavlovec, A. (1963), *J. Biol. Chem. 238*, 3717.

Petermann, M. L., and Pavlovec, A. (1966), *Biochim. Biophys. Acta 114*, 264.

Reisfeld, R. A., Lewis, U. J., and Williams, D. E. (1962), *Nature 195*, 281.

Schachman, H. K., and Edelstein, S. J. (1966), *Biochemistry 5*, 2681.

Schwartz, E. R., and Petermann, M. L. (1966), *Biochim. Biophys. Acta 112*, 119.

Staehelin, T., and Meselson, M. (1966), *J. Mol. Biol. 19*, 207.

Traub, P., Nomura, M., and Tu, L. (1966), *J. Mol. Biol. 19*, 215.

Traut, R. (1966), *J. Mol. Biol. 21*, 571.

Waller, J. P. (1964), *J. Mol. Biol 10*, 319.

Waller, J. P., and Harris, J. I. (1961), *Proc. Natl. Acad. Sci. U. S. 47*, 18.

Warner, J. (1966), *J. Mol. Biol. 19*, 383.

Yphantis, D. A. (1964), *Biochemistry 3*, 297.

Structure of the Cell Wall of *Staphylococcus aureus*. VIII. Structure and Chemical Synthesis of the Basic Peptides Released by the *Myxobacterium* Enzyme*

Derek Jarvis† and Jack L. Strominger

ABSTRACT: The basic peptide fraction obtained after hydrolysis of the cell wall of *Staphylococcus aureus* by the *Myxobacterium* enzyme has been separated into two major components, a pentapeptide and a hexapeptide. Various data indicate that the pentapeptide has the structure L-alanyl-D-isoglutaminyl-(N^{ϵ}-glycyl)-L-lysyl-D-alanine and the hexapeptide has the structure L-alanyl-D-isoglutaminyl-(N^{ϵ}-glycylglycyl)-L-lysyl-D-alanine.

The pentapeptide, and in addition the tetrapeptide lacking any glycine, have been synthesized chemically and compared to the isolated material.

A bacteriolytic enzyme from a species of *Myxobacterium* has been purified and characterized (Ensign and Wolfe, 1965, 1966). Investigations of the mode of action of this enzyme have revealed that it brings about bacteriolysis as a consequence of the hydrolysis of three linkages within the peptidoglycan of the cell wall (Tipper *et al.*, 1967a). The pentaglycine cross bridge is hydrolyzed at two positions with the liberation of both tri- and tetraglycine. Both COOH-terminal D-alanine and COOH-terminal glycine are liberated during this hydrolysis. The acetylmuramyl-L-alanine linkage in the peptidoglycan is also hydrolyzed at a slower rate. As a consequence of the hydrolysis of these three bonds a teichoic acid–polysaccharide complex, an intact polysaccharide, and a low molecular weight peptide fraction are formed. The polysaccharide fraction has been characterized and the peptide has been separated into a neutral fraction, composed of tri- and tetraglycine, and a basic fraction. The purpose of the present paper is to report the resolution of the two major components of the basic peptide fraction and the analyses of these materials. As a further proof of the structure of the isolated materials, chemical synthesis of one of these peptides as well as of the tetrapeptide common to many peptidoglycans has been carried out. The tetrapeptide has also been synthesized in parallel work carried out by Muñoz *et al.* (1966),[1] who, in addition, isolated it following enzymatic degradation of the cell walls of several different bacteria. The acetylmuramyl tetrapeptide has been obtained in small amounts during earlier studies of the cell wall of *Staphylococcus aureus* (Ghuysen *et al.*, 1965).

Materials and Methods

Analyses of total amino acids and NH_2-terminal and COOH-terminal amino acids were carried out as described by Ghuysen *et al.* (1966). Total amino acid analyses were also carried out with the Beckman-Spinco amino acid analyzer. Stepwise degradation of peptides with the Edman reagents was carried out as described by Sjöquist (1957, 1959). This technique has also been employed recently in this laboratory for the study of other peptides derived from the cell wall (Tipper *et al.*, 1967b). The dehydration and reduction of amides was carried out as described by Ressler and Kashelikar (1966). We wish to thank Dr. Ressler for the use of the facilities of her laboratory and for assistance with these analyses.

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[1] In earlier synthetic work, the protected pentapeptide with the sequence L-Ala-D-Glu-L-Lys-D-Ala-D-Ala was synthesized (Garg *et al.*, 1962; Tesser and Nivard, 1964). At about the same time both the unprotected pentapeptide and the acetylmuramyl pentapeptide were synthesized (Lanzilotti *et al.*, 1964) and shown to be identical with the material derived from the uridine nucleotide, uridine diphosphate acetylmuramyl pentapeptide. These materials differ from the compounds synthesized here and by Munoz *et al.* (1966) in lacking an amide on the α-COOH of glutamic acid and in carrying a second D-alanine residue at the COOH terminus.