bacteria are many and complex, and it is not evident whether they are more or less stable to inactivation than viral or other types of antigens. Nevertheless, these results do strike an optimistic note for further investigations. Furthermore, these studies have provided us with a model system of antigenically-active and suitably embedded bacterial cells with which to test various antibody conjugates as specific stains for electron microscopy, and such investigations are in progress.

These studies were supported in part by grants from the National Institute of Allergy and Infectious Diseases: E-1204(C4) to S. J. Singer and E-1475 to the Viral and Rickettsial Disease Laboratory.

The hospitality extended to one of us (S.J.S.) while on sabbatical leave at the Virus Laboratory at the University of California, Berkeley, is gratefully acknowledged. We are indebted to Mr. Charles Knight and Miss Jean Chin for their competent technical assistance.

* John Simon Guggenheim Memorial Foundation Fellow, 1959–60, on leave from Yale University.

† Contribution No. 1641.


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**STUDIES ON THE COMPOSITION OF THE PROTEIN FROM ESCHERICHIA COLI RIBOSOMES**

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*Communicated by John T. Edsall, November 14, 1960*

Much recent evidence indicates that protein synthesis takes place on the ribonucleoprotein particles now commonly known as ribosomes. Both in-vitro and in-vivo experiments with radioactive amino acids have produced evidence that peptide linkages are formed on ribosomes and that the growing polypeptide chains remain attached to ribosomes until their completion. A question thus poses itself: What fraction of the total protein of the ribosomes consists of growing or newly
completed polypeptide chains, and what is the chemical nature of the remainder of the ribosomal protein, which may be regarded as a structural component of the ribosomes? Calculations from the amount of bound amino acids in in-vitro experiments suggest that the growing peptide chains represent approximately 0.1 per cent of the total ribosomal protein.\(^1\)\(^2\) This figure, however, might seriously underestimate newly made protein, if some does not immediately leave the ribosomes but remains attached for an indefinite time. In the work presented here, this problem is directly attacked by examining \textit{E. coli} ribosomal protein for its amino-terminal groups. Only two major end groups are found, methionine and alanine. Ribosomal protein thus appears not to be a random sample of total cellular protein but on the contrary, a class (see below) of basic proteins which quite possibly serves the role of maintaining ribosomal RNA in a suitable configuration for protein synthesis.

There exist four main varieties of \textit{E. coli} ribosomes, with sedimentation constants of 30 S, 50 S, 70 S, and 100 S.\(^3\)\(^4\)\(^5\) Each type contains about 63 per cent RNA and 37 per cent protein. At suitable magnesium ion concentration, one 30 S and one 50 S ribosome combine to form a 70 S ribosome, while the 100 S ribosome is a dimer of two 70 S ribosomes which forms at higher magnesium ion concentration. We have examined the amino end groups of the protein from both 70 S ribosomes and its 30 S and 50 S subunits by the fluorodinitrobenzene (FDNB) method of Sanger.\(^6\)

\textit{Preparation of Ribosomal Proteins}.—The three types of particles were prepared as previously described.\(^5\) The protein fractions which were used for NH\(_2\)-terminal group analysis were prepared by the acetic acid procedure.\(^7\) Two volumes of cold glacial acetic acid were added to a cold solution of ribosomes (1–3\% by weight), and the RNA which immediately precipitated was kept in the form of a fine suspension in a teflon homogenizer. After 45 minutes at 2–4\(^\circ\), the RNA precipitate was collected by centrifugation; the supernatant solution was dialyzed in the cold to remove most of the acetic acid (as well as any free amino acids or small peptides which may have been associated with the ribosomes) and freeze-dried in order to recover the protein. In this manner, protein preparations containing about 85 per cent of the total protein of the ribosomes were obtained. The remainder of the protein was retained in the RNA precipitate and could not be extracted from it even after repeated washing with 67 per cent acetic acid. This protein fraction has since been separately isolated by another method (J.-P. Waller, unpublished data), and preliminary studies indicate that it is essentially similar in end groups, and not greatly dissimilar in amino acid composition, to the main acetic acid–soluble protein fraction.

\textit{NH\(_2\)-Terminal Group Analysis}.—The protein extracted with 67 per cent acetic acid showed a marked tendency to aggregate, with resulting insolubility over a wide range of pH. Coupling reactions with FDNB reagent\(^6\)\(^8\) were accordingly carried out in the presence of either 8 M urea or 6 M guanidine hydrochloride. In most cases, the protein (20 mg in 1 per cent solution) was allowed to react with FDNB in 0.1 M NaHCO\(_3\) containing 8 M urea (pH 8.7) at 40\(^\circ\) for four hours. The DNP-protein was precipitated with HCl, collected by centrifugation, and washed successively with N HCl, acetone, and ether. It was then hydrolyzed in a sealed evacuated tube with constant boiling HCl for 12 hours at 110\(^\circ\), and the DNP-
derivatives of its NH₂-terminal amino acids were identified and estimated according to the method of Levy. Special hydrolyses were performed for the estimation of DNP-proline and DNP-glycine.

Results.—The results obtained in a series of experiments with protein preparations from 70 S, 50 S, and 30 S ribosomes are summarized in Table 1. In all cases, alanine and methionine, the latter in slight excess, were found to be the major NH₂-terminal amino acids, and collectively they account for about 85 per cent of the total end groups found. Smaller amounts of DNP-serine and threonine and traces of DNP-aspartic and glutamic acids were also found. Although these occurred in relatively minor amounts, they were nevertheless obtained consistently and in reproducible proportions from several different preparations of 70 S ribosomes. The fact that they also occur in the proteins from both 30 S and 50 S ribosomes suggests very strongly that they represent the NH₂-terminal groups of genuine protein chains in the ribosomes. These results, in agreement with the results of quantitative amino acid analyses, also reveal a remarkable similarity between the protein moieties of the three types of nucleoprotein particles.

The possibility that the minor end groups arise through degradation of the protein, either during the isolation procedure or during its subsequent reaction with FDNB, has been considered, but the results of suitably designed control experiments do not support this view. When, for example, ribosomal protein was isolated by a procedure which involves the use of 4 M urea at pH 7.3 and 20°C, the minor end groups were found to occur in the same proportions as had been found for the protein prepared by the acetic acid procedure. There was no increase in the amounts of minor end groups when the FDNB reaction was carried out in 8 M urea at pH 8.7 and 40°C for 4 hours (i.e., standard conditions), or 12 hours, respectively. The same end group values were also obtained when the FDNB reaction was carried out in 6 M guanidine hydrochloride at pH 7.3 and room temperature for 5 hours or 12 hours respectively.

When appropriate corrections are applied to account for hydrolytic and chromatographic losses incurred during the DNP procedure, a value of about 25,000 may be calculated for the average molecular weight of the constituent protein chains in ribosomes. The calculation is based on the recoveries of the two major end groups, which were estimated by hydrolyzing the appropriate DNP-amino acid in the presence of DNP-protein (from 70 S ribosomes). For DNP-alanine and DNP-methionine (including methionine sulfoxide), the mean recovery values for two determinations were 77.1 (±0.8) per cent and 79.3 (±1.8) per cent respectively. Recoveries of the minor groups were not estimated, but they should not significantly alter the results in Table 1.

In arriving at the average molecular weight value of 25,000, we have made the following assumptions: (a) that all the protein chains possess NH₂-terminal amino acids which are free to react with FDNB, and (b) that the DNP-amino acids which we have identified and estimated represent genuine NH₂-terminal residues of the protein. Further work involving a much more comprehensive analysis of the protein is being undertaken to establish the validity of these assumptions.

The results obtained by the FDNB method were confirmed when the paper strip modification of the phenylisothiocyanate method was applied to ribosomal protein. In this manner, alanine and methionine were again found to be the major
TABLE 1

THE NH₂-TERMINAL RESIDUES OF THE PROTEINS FROM E. coli RIBOSOMES

The proportions of the NH₂ terminal groups are expressed as percentages of the total endgroups found. Results not corrected for hydrolytic and chromatographic losses (see text).

<table>
<thead>
<tr>
<th>Particle preparation*</th>
<th>70 S (C)</th>
<th>70 S (C)†</th>
<th>70 S (D)</th>
<th>70 S (E)‡</th>
<th>50 S (E)†</th>
<th>30 S (E)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₂-terminal amino acid:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alanine</td>
<td>37.9</td>
<td>36.3</td>
<td>37.2</td>
<td>36.1</td>
<td>40.0</td>
<td>38.2</td>
</tr>
<tr>
<td>Methionine</td>
<td>48.5</td>
<td>45.4</td>
<td>49.2</td>
<td>46.7</td>
<td>46.0</td>
<td>47.2</td>
</tr>
<tr>
<td>Serine, §</td>
<td>8.8</td>
<td>13.0</td>
<td>8.2</td>
<td>11.4</td>
<td>11.6</td>
<td>10.7</td>
</tr>
<tr>
<td>Threonine</td>
<td>2.9</td>
<td>3.9</td>
<td>3.2</td>
<td>3.2</td>
<td>3.8</td>
<td>1.1</td>
</tr>
<tr>
<td>Aspartic acid and glutamic acid</td>
<td>1.8</td>
<td>1.3</td>
<td>1.0</td>
<td>1.1</td>
<td>1.5</td>
<td>0.9</td>
</tr>
<tr>
<td>Lysine</td>
<td>trace</td>
<td>trace</td>
<td>1.1</td>
<td>0.9</td>
<td>1.0</td>
<td>—</td>
</tr>
<tr>
<td>Weight of protein/mole of end groups (uncorrected)</td>
<td>35,600</td>
<td>30,200</td>
<td>33,200</td>
<td>32,400</td>
<td>31,400</td>
<td>31,900</td>
</tr>
<tr>
<td>Weight of protein/mole of end groups (corrected) ††</td>
<td>27,800</td>
<td>23,600</td>
<td>25,900</td>
<td>25,300</td>
<td>24,500</td>
<td>25,000</td>
</tr>
</tbody>
</table>

* Letters C, D, and E refer to particles obtained from different E. coli preparations.
† Reaction in the presence of 6 M guanidine-HCl and KHCO₃ (pH 7.3) for 5 hours at 40°. All other reactions in 8 M urea and NaHCO₃ (pH 8.7) for 4 hours at 40°.
‡ Each sample analyzed in duplicate. The 50 S particle preparation used was virtually pure, while the 30 S preparation was contaminated by approximately 20% of 50 S.
§ Values uncorrected for minor contamination by methionine sulfoxide. By rechromatography of the DNP-"serine" spot in the solvent benzene-acetic acid-water (1:1:1), the average serine value for protein from 70 S particles was established as 8% of the total end groups. Higher values were accompanied by a corresponding decrease of the methionine and can be attributed to methionine sulfoxide.
** The lower yield is attributed to the smaller amount of protein (10 mg instead of the usual 20 mg) available for analysis.
†† Approximate values, derived by using an average correction factor of 0.78, based on the recoveries of the 2 major DNP-end group (see text).

end groups, while serine, threonine, and aspartic and glutamic acids were also present in smaller amounts. The average molecular weight of the protein chains (calculated from the optical density at 268 mµ of the phenylthiohydantoin derivatives of the NH₂-terminal amino acids) was found to be 26,000, a value in good agreement with that obtained by the FDNB method.

Fractionation of the protein from E. coli ribosomes (70 S): In order to further investigate the composition of E. coli ribosomal protein in the light of the NH₂-end...
group results, the protein has been submitted to various fractionation procedures, including starch gel electrophoresis\textsuperscript{13} and ion-exchange chromatography.

When acetic acid–soluble protein from ribosomes or intact 70 S ribosomes are submitted to starch gel electrophoresis in the presence of 6 M urea at pH 5.6, a complex pattern of at least 20 bands appears on staining with amido black (Fig. 1). The possibility that aggregation may be responsible for the complexity of this pattern has been considered, but it appears most unlikely on the basis of the following results:

(a) When acetic acid–soluble protein, adsorbed on a column of carboxymethyl-cellulose\textsuperscript{13} in a solution of 0.01 N HCl in 6 M urea (pH 3.32) at 2° is eluted by progressively lowering the pH to 2.50, the protein can be recovered in two distinct chromatographic peaks, I and II. Starch gel electrophoresis in 6 M urea at pH 5.6 of the material from the two peaks (recovered after extensive dialysis against water and freeze-drying) shows a separation into two distinct sets of bands, which to-

![Figure 2](image)

**Fig. 2.**—Starch gel electrophoresis in 6 M urea at pH 5.6 (for conditions, consult Fig. 1) of the materials from peaks I and II obtained when acetic acid-soluble protein is fractionated on carboxymethylcellulose under conditions described in the text. (a) is protein from peak II (2.5 mg); (b) is protein from peak I (2.5 mg); (c) is unfractionated acetic acid–soluble protein (3.5 mg). The bands in the overlapping region of (a) and (b) appear to be two distinct sets of bands, the combination of which accounts for the intense staining in the same area of the unfractionated protein. This apparent is ascribed to the influence of the carboxyl groups of the protein. While these were only partly ionized during chromatography in acid pH, they are fully ionized during electrophoresis at pH 5.6.

gather account for all the bands observed in the unfractionated protein (Fig. 2). The more basic bands are from the material in peak II, in accordance with its chromatographic behavior on carboxymethyl-cellulose.

(b) As a further control, the material eluted from carboxymethyl-cellulose in the system described above was subdivided into ten arbitrary fractions from successive portions of the effluent in the order of increasing acidity. The protein from each of these fractions was isolated by extensive dialysis against water and freeze-drying and was submitted to starch gel electrophoresis in 6 M urea at pH 5.6. In spite of a considerable overlap of the bands from immediate neighboring fractions, a clear separation into small groups of bands, with increasingly basic properties from fraction 1 to 10, was observed. There was no apparent evidence of re-aggregation.

The proteins from peaks I and II, derived from chromatography on carboxymethyl-cellulose as described above, were separately examined for NH\textsubscript{2}-terminal groups by the FDNB method. Alanine and methionine end groups were present in
both fractions. The results clearly indicate that in ribosomes the protein chains possessing an NH$_2$-terminal methionine (or alanine) residue are not all chemically identical.

Discussion and Summary.—NH$_2$-terminal group analyses on E. coli ribosomal protein indicate the presence of two major end groups: methionine and alanine. The results are consistent with the view that ribosomal protein is not a random sample of total cellular protein but, on the contrary, a class of basic proteins which quite possibly serves the role of maintaining ribosomal RNA in suitable configuration for protein synthesis.

The constituent protein chains of ribosomes, with an average molecular weight of 25,000, thus fall into two major classes: those chains with methionine as NH$_2$-terminal residue and those with alanine. From the results of fractionation experiments, it is further apparent that within each of these two major classes not all chains are chemically identical. The extent and significance of the chemical differences between chains possessing the same NH$_2$-terminal residue are now being investigated. It is of interest to note that, in this respect, E. coli ribosomal protein bears a striking resemblance to calf thymus histone, where a simple amino end group picture (with alanine and proline accounting for over 90 per cent of the end groups)$^{14}$ is likewise accompanied by a complex protein composition.$^{15}$

We wish to thank Drs. J. T. Edsall, J. D. Watson, and, A. Tissières for their interest and for helpful discussions. We are indebted to the National Institutes of Health (Grant H-3169) and the National Science Foundation (Grants G-9116 and G-10895) for research support.

$^*$ Member of the Scientific Staff of the Medical Research Council of Great Britain and Research Fellow in Biology, Harvard University, 1959.


$^2$ Tissières, A., D. Schlessinger, and F. Gros, these PROCEEDINGS, 46, 1450 (1960).


$^9$ Spahr, P. F. (Unpublished results).

$^{10}$ Waller, J.-P. (Unpublished results).


