The Absence of Histone in the Bacterium *Escherichia coli*

I. Preparation and Analysis of Nucleoprotein Extract

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ABSTRACT

The deoxyribonucleic acid (DNA) from *Escherichia coli* has been isolated as an extract containing about 50 per cent by weight protein. The protein component differs both in composition and chemical behaviour from histone which occurs in combination with the DNA in most cells of higher organisms. Although this result suggests the absence of histone-like protein, it is not clear whether the bacterial protein found is naturally bound to the bacterial DNA in the cell or becomes attached to the DNA during the course of isolation.

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It is now fairly well established that the deoxyribonucleic acid (DNA), in the cells of all higher organisms that have been closely examined, is complexed with protein (1). Invariably the protein part of the complex has a high content of arginine and lysine residues, and it is commonly believed that the stable association with DNA is due to the interaction between the positively charged amino acid side chains and the negatively charged DNA phosphate groups. In addition to the basic proteins there are less certain indications that the DNA may also be combined to a varying degree with neutral or acid proteins, which have been collectively termed "residual protein" (2). However, the manner of attachment of "residual protein" to DNA remains obscure and there is still some question as to whether it is attached to DNA in the cell or only in isolated preparations.

It has been known for some time that DNA preparations containing protein may be obtained from bacterial organisms (3–5). However, little effort has been devoted to the study of these deoxyribonucleoprotein extracts as such. We have sought to investigate the state of DNA isolated from bacteria: firstly, to see to what extent the DNA is associated with protein; secondly, to investigate the chemical nature of the protein; and thirdly, to examine the mode of linkage between the DNA and the protein.

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The choice of *Escherichia coli* was due to several factors; it has been intensively studied by both biochemists and geneticists and it is particularly easy to grow and handle in large quantities.

In the initial formulation of our method of preparation we considered that in order for our results to have the greatest possible significance we must avoid the use of deproteinizing agents. This imposed great hazards, for in the normal course of making a DNA preparation the presence of deproteinizing agents would inactivate interfering degradative enzymes. Our main precautions against enzyme attack involved the use of extensive washing with versene, high pH, low temperature, and the fastest possible working time.

EXPERIMENTAL

Preparation of Nucleoprotein.—A culture containing 14 litres of *E. coli* strain B-2 (6) was grown in synthetic M-9 medium (7) to an O.D. (650 mμ, 1 cm.) of about 0.75. The bacilli were harvested by spinning down in a Sharples centrifuge and were resuspended in 5 litres of a standard saline medium (0.024 M versene pH 8 + 0.075 M NaCl). The suspension was collected again in the Sharples and the process of washing was then repeated twice with the saline medium and once with distilled water. All washing and further preparative work was done at 5°C. The final sediment from the Sharples was stored in a plastic bag on dry ice until required. The frozen sediment was allowed to thaw at 5°C for 2 hours and then combined with two volumes of fine grain alumina powder (activated alumina type "O," Peter Spence and Sons Ltd., Widnes, England) in

a mortar, adding sufficient water to make a thick paste. The paste was ground vigorously for 12 minutes and resuspended in 400 cc. of distilled water with stirring for 15 minutes. The alumina was removed by 5 minutes spinning in an International centrifuge at 3,000 *g* and the unbroken cell fragments and heavier cell components were spun down in 4.5 hours at 30,000 *g* in a Spinco ultracentrifuge. The sediment from this centrifugation was discarded and 0.2 volumes of standard saline medium were added to the supernatant from the above centrifugation and the solution was then spun for 16 hours at 30,000 *g*. The pellets in the bottom of the centrifuge tubes were found to contain all of the DNA from the first supernatant and this was dispersed in distilled water by stirring for 2 hours, after which an equal volume of saline medium (0.048 M versene pH 8 + 0.15 M NaCl) was added to make a total volume of 400 cc. This was spun as before and the process of washing and centrifuging was repeated once again in the same way and once using water only. The final water washed sediment contained about 60 mg. of "purified DNP." The yield seems to vary greatly with the amount of grinding in the first step and in one case a yield of 60 per cent was obtained with the same final N/P ratio (nitrogen/phosphorus weight ratio). In general we contented ourselves with a 30 per cent yield because we did not wish to expose our material to excessive grinding.

Chemical Analysis.—Nitrogen, was determined by a micro-Kjeldahl method using a Cu-Se catalyst and a digestion period of 16 hours. *Phosphorus,* was determined according to reference 8. *Nucleic acids,* RNA was estimated by Bial's orcinol test and DNA by the Dische test, both used as described by Dische (9).

Amino Acid Analysis.—In view of the possibility that attempts to separate the protein quantitatively from the nucleic acid might consume a great deal more material than could be readily prepared, it was considered worth while to estimate the amino acids in a hydrolysate of the intact nucleoprotein. To this end 25.8 mg. (air-dry weight) of bacterial DNP was hydrolyzed with 4 cc. of 6 N HCl at 110°C. for 26 hours. The hydrolysate was centrifuged to remove "insoluble humin," and the supernatant, together with water washings of the sediment, was made up to 10 cc. with water. Two 4 cc. samples of this solution (each equivalent to 10.3 mg. of nucleoprotein) were taken for the determination of amino acids according to the method of Moore and Stein (12). The amino acid peaks found in the digest were readily identified, and no unusual peaks appeared. Glycine is high owing to the formation of this substance from purines during hydrolysis (13). Methionine has become partially oxidized, and the value given is derived from the sum of the methionine and methionine sulfoxide peaks. Cystine in proteins is usually oxidized to cysteic acid when the hydrolysis is performed in the presence of carbohydrates. The value given is derived from the peak at the hold up volume of the column, which may contain substances other than cysteic acid. The value is therefore an upper limit. Serine and threonine have not been corrected in any way for breakdown during hydrolysis. The values given are probably 10 and 5 per cent low respectively. The recoveries of the remaining amino acids are probably not affected by the carbohydrate present, since good recoveries under these conditions were obtained by Dustin (14) and one of us (15). Some of the ammonia found must certainly come from degradation of the nucleic acids and because of this it was not possible to estimate the number of amide side chains. The result is that it is not possible to tell from this analysis whether the protein at neutral pH is acidic or basic.

TABLE I

Amino Acid Composition of Bacterial DNP (I), Total Protein of E. coli (II), and Total Thymus Histone (III)

The amino acid values have been calculated as the number of residues in every 100 residues.

	I	II (10)	III (11)
Glycine	11.8	7.8	8.5
Alanine	10.1	12.7	13.7
Valine	6.9	5.5	6.2
Leucine	8.6	7.9	7.9
Isoleucine	5.3	4.6	4.4
Phenylalanine	3.1	3.3	2.1
Proline	4.2	4.6	5.1
Serine	4.3	6.1	4.9
Threonine	5.7	4.7	5.3
Aspartic acid	10.0	9.9	4.85
Glutamic acid	11.5	10.5	8.3
Arginine	6.1	5.3	8.4
Histidine	1.7	0.97	2.0
Lysine	6.3	7.0	14.9
Methionine	1.6	3.4	1.0
Cystine/2 + cysteine	0.6	1.7	<0.1
Tyrosine	2.3	2.1	2.4
Diaminopimelic acid	—	0.44	—
Tryptophan	—	1.04	—

RESULTS

The product of this preparation had an N/P ratio of 3.60 ± 0.2. No ribonucleic acid was detectable by the Bial orcinol test; this shows that more than 90 per cent of the nucleic acid present was DNA. A comparison of the Dische reaction with the phosphorus content indicated that at least 95 per cent of the phosphorus was accountable as DNA. From this it follows, together with the N/P ratio of 3.6 and the N/P ratio from DNA

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of 1.66 (16), that about 46 per cent of the total nitrogen in the samples is DNA-nitrogen. From the recoveries of the amino acids in the analysis, it was estimated that some 55 per cent of the total nitrogen in the sample was protein-nitrogen. Thus the nitrogen of the samples appears to be accounted for in terms of protein and DNA. The protein was analysed for amino acids and the results of this analysis are presented in Table I, together with the average amino acid content of the total *E. coli* protein (10) and total thymus histone (11).

Our efforts turned at this point to an examination of the linkage between the DNA and the protein. We observed that the N/P ratio of 3.60 was reproducible in different preparations and did not change significantly on further washing or prolonged dialysis. When the restriction on the use of deproteinizing agents is relieved it is possible to remove the protein in various ways. The most successful combination for protein removal appears to be a combination of strong salt and surface active agent, such as chloroform-amyl alcohol or sodium dodecyl sulfate (3–5).

DISCUSSION

Examination of Table I indicates that the bacterial DNP does not contain high proportions of basic side chains which are characteristic of the histones. Comparison of the nucleoprotein with total *E. coli* protein indicates a striking similarity in composition. These results suggest that there is no appreciable quantity of histone complexed with the bacterial DNA unless it has been lost during the isolation procedure. Although this latter possibility seems most unlikely, we have no proof that it is not so.

At present we are not in a position to say whether this DNA protein extract is an artifact or a complex found in the intact bacteria. Nevertheless, there is a good deal of evidence that the DNA is not chemically free in the intact bacteria. The light scattering molecular weight on purified preparations of *E. coli* DNA is about 9 million (17). From this molecular weight and a rough estimate by Dr. G. L. Brown of the DNA content per *E. coli* cell one may calculate that there are several hundred molecules of DNA in each bacterial cell. If we couple this knowledge with the genetic finding that *E. coli* appears to contain a single linkage group (18), it suggests that the DNA molecules are held together and organized by some sort of chemical linkage. Added weight to this conviction results from the observations of Robinow (19) and others that several species of bacteria including *E. coli* contain a dense Feulgen-positive nuclear body which appears to be composed of about equal amounts of DNA, RNA, and protein (20).

Recently the isolation of a desoxynucleoprotein from *E. coli* was reported by Palmade *et al.* (21). The *E. coli* were grown under conditions conducive to the formation of protoplasts rather than whole cells and the method of extraction of the nucleoprotein differed from the one used here. It is naturally of the greatest interest to see if the two very different procedures result in the same final product. The nucleoprotein isolated by Palmade has an N/P ratio of 3.64 and a paper chromatogram of an amino acid hydrolysate gave a pattern resembling the paper chromatogram of histone. These results led to the suggestion that the protein part of the complex may be a histone. The N/P ratio of Palmade is in agreement with ours suggesting that the two nucleoprotein extracts are similar. However, the more detailed and quantitative amino acid analysis of our product indicates that although there is a superficial similarity to histone (compare columns I and III in Table I) ordinary histone contains about twice as many basic residues. The resulting lower basicity of the bacterial protein is undoubtedly the primary factor responsible for the loosely woven structure which the bacterial nucleoprotein appears to form in contrast to nucleohistone (see following paper).

It is difficult to judge if the type of DNP found for *E. coli* would be obtained if the same isolation procedure was applied to other bacteria, since few detailed investigations have been made. Chargaff (5) has reported the isolation of purified *avian tubercle* DNP with an N/P ratio of 3.8. The method of preparation was quite different and involved the use of concentrated salt solutions but the close agreement with our N/P value of 3.6 suggests that the material may be similar. Unfortunately a fuller comparison is not possible. The only other observation which seems immediately pertinent to this study is that ionic strength does not seem sufficient for removing the protein from the bacterial DNA (3, 4) although the amount of protein remaining after treatment seems to vary a great deal in different species. This again contrasts with the behavior of nucleohistone, from which it has been shown that ionic strength

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is capable of dissociating the vast majority of the protein from the DNA.

The apparent absence of large amounts of basic proteins on the DNA of the rapidly dividing cells of *E. coli* is rather surprising in view of its omnipresence in the cell nuclei of higher organisms. This is good reason for suggesting that the biologically active form of DNA is free of basic proteins, and that such proteins do not directly assist the nucleic acid in the transfer of genetic information.

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