Proc. Nat. Acad. Sci. USA
Vol. 72, No. 9, pp. 3428–3432, September 1975
Biochemistry

Characterization of a novel, low-molecular-weight DNA-binding protein from *Escherichia coli*

(DNA protein interaction/transcription stimulation/histone-like/electron microscopy)

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Communicated by André Lwoff, June 30, 1975

ABSTRACT **A low-molecular-weight (7000), heat-stable protein—HU—that stimulates transcription of bacteriophage λ DNA by *E. coli* RNA polymerase was purified from *E. coli* extracts using affinity chromatography on DNA-cellulose. HU binds to native DNA, resulting in an apparent thickening of the DNA chains as revealed by electron microscopy. Contrary to DNA unwinding proteins, it causes no destabilization of the double helix. HU differs from previously described transcription factors (H_1, D, etc.) and from the low-molecular-weight ω subunit of the RNA polymerase. By its amino-acid composition and characteristics, HU displays an interesting resemblance to some eukaryotic histones, such as H2B and H1.**

A variety of low-molecular-weight proteins from *Escherichia coli* that stimulate RNA synthesis *in vitro* have been characterized (1–8). The heat-stable protein, H_1 (1, 2), was shown to enhance λ-*lac* DNA transcription by *E. coli* RNA polymerase (3) while causing reduction of ribosomal RNA synthesis in an *E. coli* DNA-dependent system (4). A heat-stable protein, the D factor, was reported to increase the specificity of λ-DNA transcription by the *E. coli* polymerase (5). Another class of small, heat-stable proteins has also been described which stimulates *in vitro* the replication of RNA bacteriophage (6, 7). That these small protein factors could act by locally affecting the stability of nucleic acid secondary structure, hence favoring or inhibiting the action of polymerases, has already been suggested (1, 2, 5, 8), and it has been proposed that some of these entities, such as the H_1 and the D factors, could represent the prokaryotic counterpart of eukaryotic nuclear proteins (3, 5–8).

In the frame of this hypothesis, we have undertaken a more systematic analysis of DNA binding proteins by means of affinity chromatography on DNA-cellulose columns. We report, here, the purification from *E. coli* extracts of a small, heat-stable protein—HU—that stimulates transcription of bacteriophage λ-DNA and displays by its amino-acid composition and physicochemical behavior some properties characteristic of eukaryotic histones.

MATERIALS AND METHODS

Bacterial Strains and Growth of Cells. *E. coli* U 13, an RNase I^- strain (originated from W. Salser), or MRE 600 (from the Pasteur Institute collection) was grown at 37° in mineral medium 63 (9) supplemented with 0.3% casamino acids, 0.1% yeast extract, and 1.0% glucose. Cells were harvested in late logarithmic phase, washed, and stored at −20°.

Reagents. [^{3}H]Methyl-thymidine (25 Ci/mmol) and [^{3}H]UTP (15 Ci/mmol) were purchased from CEA (Saclay, France). Unlabeled nucleoside triphosphates were from Sigma; pancreatic deoxyribonuclease I and calf thymus DNA were from Worthington Biochemical Corp.

Bacteriophage, DNA Preparation, and Transcription Studies. DNA was obtained from CsCl purified λcI857S7 bacteriophages after Pronase digestion and phenol/sodium dodecyl sulfate ($NaDodSO_4$) extraction. The DNA was extensively dialyzed against 10 mM Tris·HCl (pH 7.5), 1 mM EDTA, then against 10 mM Tris·HCl (pH 8.1), 0.5 mM EDTA. Labeled phage DNA was prepared after thermal induction of *E. coli* W3350 thy^- (λcI857S7) grown in the presence of [^{3}H]thymidine. (The lysogenic strain was a gift from Ph. Kourilsky.) RNA polymerase, purified according to Burgess (10) by ammonium sulfate fractionation followed by a DEAE-cellulose chromatography and two glycerol gradients, was a generous gift from B. Lescure, except for the preparation mentioned in the *text*. A polyacrylamide gel analysis of both preparations is shown in Fig. 1. The transcription mixture (0.1 ml) contained 20 mM Tris·HCl (pH 7.9), 10 mM $MgCl_2$, 60 mM KCl, 0.1 mM EDTA, 0.5 mM dithiothreitol, 0.2 mM ATP, CTP, GTP, and 0.2 mM [^{3}H]UTP (40 μCi/μmol). The concentrations of DNA, RNA polymerase, and HU protein were as indicated in the legends of figures. After 10 min of synthesis at 37°, the trichloroacetic acid-precipitable material was collected on Millipore filters. Radioactivity was measured by an Intertechnique scintillation counter.

Gel Electrophoresis. Proteins were analyzed by $NaDodSO_4$ gel electrophoresis (11) either on a 15% polyacrylamide gel or, whenever fine comparative analysis was necessary, on gels containing 10–25% linear gradient of acrylamide as described by Studier (12), the mono- to bisacrylamide ratio being 30:0.8. Molecular weights were determined in 15% gels using appropriate standards.

Purification of HU Protein. An extract from 200 g of *E. coli* cells was prepared as described by Alberts and Frey (13) for T_4 infected cells. The extract was made 10% glycerol in the following buffer: 20 mM Tris·HCl (pH 8.1), 50 mM NaCl, 5 mM EDTA, 0.5 mM dithiothreitol, and was pumped through a DNA-cellulose column. The column contained 90 ml packed volume of double-stranded calf thymus DNA-cellulose, that had been equilibrated with the same buffer. [Phenol deproteinized calf thymus DNA was used to prepare DNA-cellulose by the combined methods of Litman (14) and Alberts (15) as described by L. Reichart (16): cellulose powder was added to a solution of native DNA and lyophilized. The lyophilized material was suspended in absolute ethanol and UV irradiated.] Stepwise elutions were made in buffer previously described by increasing ionic strength from 0.05 M to 2.0 M NaCl at a flow rate of 50 ml/hr. The different fractions eluted from the column were tested for their stim-

Abbreviation: $NaDodSO_4$, sodium dodecyl sulfate.

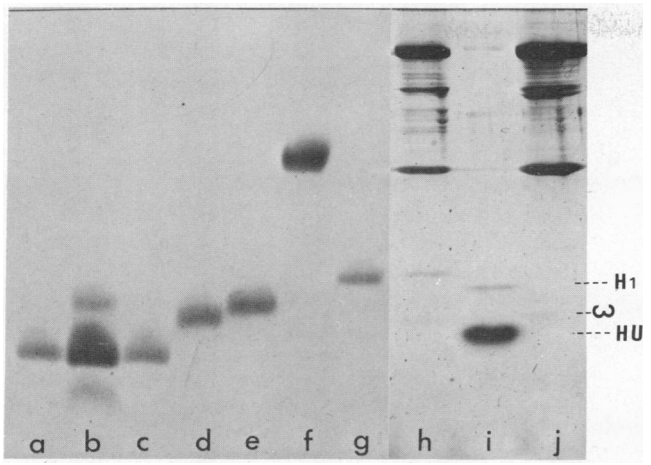

FIG. 1. Polyacrylamide/NaDodSO$_4$ gel electrophoresis. *15% gels*: (a) HU, (b) initiation factor IF1, (c) HU, (d) cytochrome *c*, (e) egg white lysozyme, (f) histone H1, (g) H_1 factor. *10–25% gradient gels*: (h) RNA polymerase purified from extract deprived of HU (see *text*), (i) HU + H_1 factor, (j) RNA polymerase purified according to Burgess (10).

ulatory activity on λ-DNA transcription. Such an activity was found to accompany the protein fractions eluted at 0.4 M NaCl. When analyzed on NaDodSO$_4$/polyacrylamide gels, this fraction contained a major band with a molecular weight of 10,000 and several minor bands of higher molecular weight. This 10,000-dalton protein was observed by Sigal *et al.* (17) during the purification of the *E. coli* unwinding protein. To pursue the purification, the 0.4 M eluate from the DNA-cellulose column was concentrated by ultrafiltration and dialyzed against 20 mM Tris·HCl (pH 8.1), 1 mM EDTA, 0.5 mM dithiothreitol, 0.3 M KCl containing 10% glycerol. The concentrated fraction (1 ml) was applied to a Sephadex G-100 column (2.5 cm × 45 cm), and fractions were collected with a flow rate of 6 ml/hr. The column effluent was recorded at 240 nm. The 10,000-dalton protein was eluted as a symmetric peak with a degree of purity, according to polyacrylamide gel, that exceeded 95%. The transcription stimulatory activity was found to be associated with this peak. We shall refer subsequently to this protein as the HU protein. Calibration of the column with dextran blue, alkaline phosphatase, peroxidase, cytochrome *c*, and Dnp-lysine permits an estimated molecular weight of 20,000 for native HU. To remove trace contaminations from the Sephadex fractions, the HU eluate was concentrated, dialyzed against 20 mM Tris·HCl (pH 8.1), 1 mM EDTA, 0.5 mM dithiothreitol, 60 mM KCl, and passed through a DEAE-cellulose column equilibrated with the same buffer. HU protein appeared in the flow-through fraction. At this stage, no DNase or RNase activities were detected, and only one band was observed on polyacrylamide gel.

RESULTS

Characteristics of HU protein

After the purification step on DEAE-cellulose column, HU is pure as judged by polyacrylamide gel. From its behavior on Sephadex gel filtration, its molecular weight can be estimated to be of the order of 20,000. Since it migrates as a 10,000-dalton component during electrophoresis under denaturing conditions (Fig. 1), we conclude that it exists in its native form as a dimer. This conclusion is supported by the fact that after treatment with a crosslinking agent (suberimidate) it gives a single band corresponding to a 20,000 molecular weight component on NaDodSO$_4$/acrylamide gels (result not shown). HU protein appears different from H_1 factor (a gift from A. Spassky) since the latter has a molecular weight of 16,000 (Fig. 1); moreover and contrary to H_1, its electrophoretic mobility is not affected by 2-mercaptoethanol reduction. Finally, no crossreaction was observed between H_1 and HU using an immunodiffusion test with an anti-H_1 antiserum (a gift of M. Jacquet). HU differs also from another small *E. coli* polypeptide, the ω subunit of the RNA polymerase (18). The Burgess purification technique was applied to an *E. coli* extract from which HU had been removed by DNA-cellulose chromatography, and the resulting RNA polymerase displayed the same ω content as the one prepared from a classical extract (Fig. 1). Moreover, the electrophoretic mobilities of HU and ω differ when analyzed on NaDodSO$_4$/polyacrylamide gel, as illustrated in Fig. 1. From the purification yields, we deduced that an *E. coli* cell contains a minimal number of 10,000 copies of HU protein.

Table 1. Amino-acid composition of HU protein

Amino acid	HU (no. of residues)	HU (mol %)	H2B (mol %)	H1 (mol %)
Lysine	9–10	14.0	14.1	26.8
Histidine	1	1.5	2.3	0
Arginine	3–4	5.1	6.9	1.8
Aspartic acid	5–6	8.1	5.0	2.5
Threonine	4	6.0	6.4	5.6
Serine	3	4.4	10.4	5.6
Glutamic acid	6–7	9.6	8.7	3.7
Proline	2	3.0	4.9	9.2
Glycine	5	7.4	5.9	7.2
Alanine	11	16.3	10.8	24.3
Cysteine	0	0	0	0
Valine	4	6.0	7.5	5.4
Methionine	1	1.5	1.5	0
Isoleucine	4	6.0	5.1	1.5
Leucine	4–5	6.6	4.9	4.5
Tyrosine	0	0	4.0	0.9
Phenylalanine	2	3.0	1.6	0.9
Tryptophan	0	0	0	0
NH_3	10	—	—	—
Lys/Arg	—	2.7	2.01	14.88

Lyophilized samples of HU were hydrolyzed at 110° in 0.5 ml of 6 M HCl for 24, 48, and 72 hr. The analysis was performed in a Technicon autoanalyzer. The results are the average of eight determinations. Molar ratios were calculated relative to phenylalanine. Absence of tryptophan and cysteine was established as described in the *text*. The compositions of histones H2B and H1 are from Johns (22, 23).

Amino-acid composition

The amino-acid composition of HU was determined on independent preparations from two different *E. coli* strains using two different procedures of extraction (one of which is the one described here, the second one is unpublished). The result shown in Table 1 indicates that HU is a lysine- and alanine-rich protein which lacks cysteine, tyrosine, and tryptophan residues. No SH groups were detected in reduced and denatured protein with Ellman's reagent [5,5′-dithio-bis-(2-nitrobenzoic acid)] (19). Lack of tryptophan was deduced from the absence of the fluorescent emission specific for this amino acid and absence of 280 nm absorbance. The absence of tyrosine and tryptophan is reflected in the UV absorption

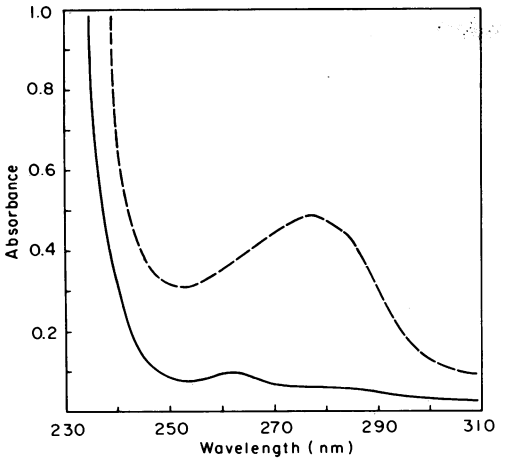

FIG. 2. Ultraviolet absorption spectrum of HU protein. HU (650 μg/ml) and aspartokinase homoserine I dehydrogenase I (770 μg/ml) were dialyzed against 20 mM potassium phosphate buffer (pH 7.0), 100 mM KCl and their UV absorption spectra were recorded. The concentration of HU was measured by microbiuret titration with the aspartokinase (a gift from P. Truffa-Bachi) as the standard. HU—; aspartokinase - - -.

spectrum, which lacks the absorbance peak at 280 nm (Fig. 2). The concentration of the protein was calculated from the amino-acid analysis. A good correlation of this value was obtained with a biuret titration (20).

From the number of amino-acid residues, a minimum molecular weight of 7000 can be calculated, a value somewhat different from that of 10,000 obtained from Na-DodSO$_4$/acrylamide electrophoretic measurements. A similar discrepancy between the molecular weight determinations deduced from the analysis and from electrophoretic mobility studies has already been reported in the case of histone H1, which on gel, behaves as a 30,000-dalton protein, whereas the known molecular weight is 20,000 (21).

A comparison of the amino-acid composition of HU with those of H2B and H1 histones (22,23) is presented in Table 1. It is clear that HU presents many similarities with H2B: identical lysine content and a similar lysine to arginine ratio. However, the serine, glycine, and alanine contents are close to those of H1 histone. As would be expected from its amino-acid composition, isoelectric focusing on gel indicates that HU is a basic protein with a pI around 8.75.

HU effect on λ DNA transcription

HU exerts a stimulatory effect on λ DNA transcription, the extent of which varies with the respective proportions of DNA template, enzyme, and factor, as well as with the ionic strength of the incubation medium. Fig. 3a shows that in the presence of a fixed amount of RNA polymerase and for varying amounts of template, initial rates of λ DNA transcription are considerably greater if HU is present. An optimal stimulation of 5- to 15-fold was observed at a DNA to enzyme weight ratio of 4, which roughly corresponds to 15 RNA polymerase molecules per DNA chain. Fig. 3b illustrates the dose response curve when increasing amounts of HU are present in the system at this optimal DNA to enzyme ratio. Maximal stimulation is observed for a stoichiometric weight ratio of HU to DNA. Independent kinetic studies have shown that HU essentially increases the initial rate of [^{3}H]UTP incorporation. HU effect on DNA transcription is not affected if the factor is preincubated for 10 min at 100°, showing that HU is a thermostable protein.

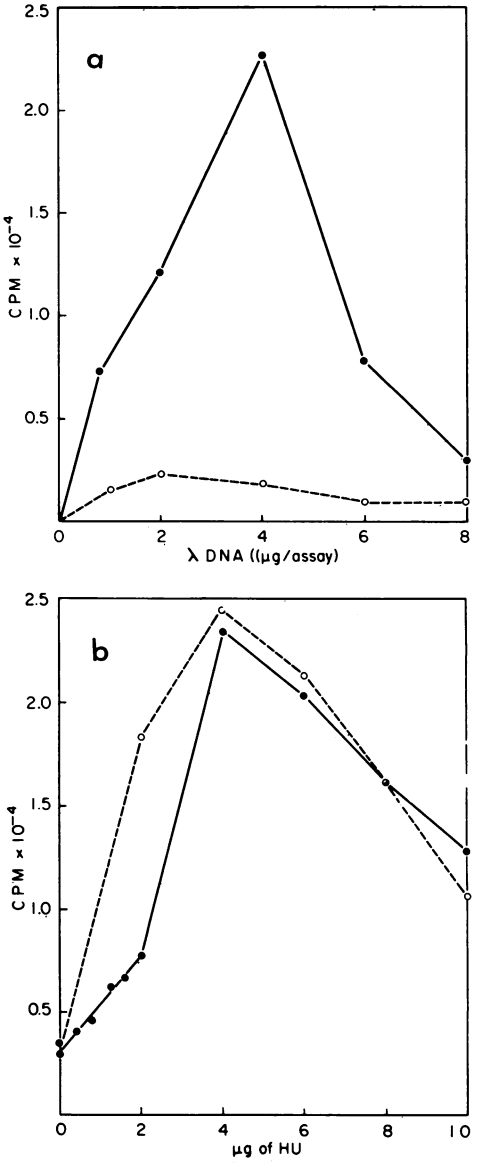

FIG. 3. Stimulation of λ DNA transcription by HU protein. (a) Increasing amounts of λ DNA were mixed in absence or in presence of 4 μg of HU protein with 1 μg of RNA polymerase under the ionic conditions described in *Materials and Methods*. To provide synchronous initiation, the reaction was started by addition of the nucleoside triphosphate. RNA synthesis in the presence of HU (●), in absence of HU (○). (b) Four micrograms of λ DNA were mixed with 1 μg of RNA polymerase in presence of increasing amount of HU protein. Native HU protein (●); HU protein preincubated for 10 min at 100° (○).

That the HU effect on transcription appears to be at the template rather than enzyme level, is suggested by the fact that the amount of protein required for optimal stimulation doubles when the DNA to enzyme ratio is increased by a factor of two (data not shown).

HU-DNA interaction

Further proof that HU must be acting by modifying the template rather than the enzyme comes from studies showing that HU readily binds λ DNA. Three different approaches were used to monitor this capacity.

The filter binding assay used in the study of various DNA-protein interactions was used. Whereas native λ DNA passes

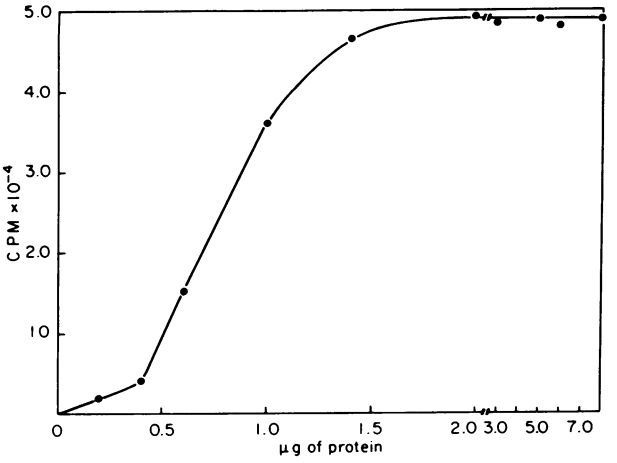

FIG. 4. Retention of native λ DNA on cellulose nitrate filters. Four micrograms of λ [^{3}H]DNA (1.2 × 10^7 cpm/mg) were mixed with increasing amount of HU protein in 0.1 ml of binding solution containing 20 mM Tris·HCl (pH 7.9); 0.1 mM EDTA; 60 mM KCl. After 10 min of incubation at 37°, the samples were diluted to 1.0 ml with binding buffer containing 5% dimethylsulfoxide and 50 μg/ml of bovine serum albumin. The samples were then filtered through 24 mm Schleicher and Schuell nitrocellulose membranes and rinsed with 1.0 ml of the same solution.

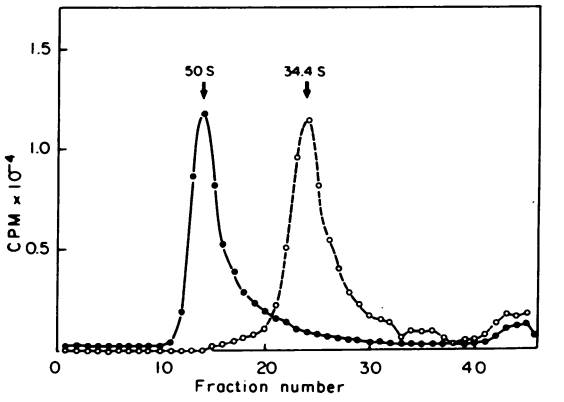

FIG. 5. Effect of HU on sedimentation of λDNA. One microgram of λ [^{3}H]DNA (7.8 × 10^7 cpm/mg) was incubated in absence or in presence of 1 μg of HU protein in the binding buffer described in Fig. 4. After 10 min of incubation at 37°, the samples (0.1 ml) were layered on a linear 10–30% glycerol gradient in the same buffer (a 0.5-ml cushion of 50% glycerol was used). Sedimentation was performed at 49,000 rpm at 4° for 2 hr in a SW 50.1 rotor with L3-50 centrifuge. DNA alone (○); DNA in presence of HU (●).

through nitrocellulose filter, the λ DNA-HU complex is retained. When a constant amount of λ [^{3}H]DNA is mixed with increasing amounts of HU protein, the fraction of DNA retained reaches 100% with a protein to DNA weight ratio of 0.5 (Fig. 4). The sigmoid appearance of the binding curve may indicate a cooperativity in the binding process. Another interpretation is that a minimal number of bound HU molecules is necessary in order to retain the DNA on the filter. Further experiments will be needed to distinguish between these two alternatives.

To further explore the formation of a λ DNA–HU complex as well as its stability, the following experiment was performed. λ [^{3}H]DNA alone and a HU λ–DNA complex in a weight ratio of 1 to 1 were sedimented through a 10–30% linear glycerol gradient. As is shown in Fig. 5, the sedimentation coefficient of λ DNA was increased from 34.4 S to 50 S by the presence of HU. The absence of trailing in the sedimentation profile of the DNA in the presence of HU indicates the formation of a stable complex between the two molecules.

To examine the nature of the interaction, the complex was analyzed by electron microscopy. To facilitate these studies, λ DNA was cleaved by endonuclease Eco RI (a gift from M. Yaniv) and HU was added to the mixture of fragments in a weight ratio of 1 to 1 in the presence of 0.4 M KCl in binding solution. The complex was dialyzed against binding solutions containing successively 0.3 M, 0.2 M, 0.1 M, and 0.06 M KCl in order to decrease progressively the ionic strength to 60 mM KCl. DNA alone was treated in the same manner. In order to visualize the complex, carbon-coated grids were activated by discharge in amylamine atmosphere according to Dubochet *et al.* (24). Samples were diluted in 10 mM Tris·HCl (pH 7.5), 1 mM EDTA, applied to the grids, and stained with aqueous uranyl acetate. Grids were examined directly in bright or dark field electron microscopy or rotary shadowed with Pt–Pd. As can be seen in bright field (Fig. 6a and b) the DNA–HU complex appears much thicker than native DNA, with variation of the thickness along the molecules. In the presence of HU, the DNA tends to form small loops and hairpin structures, probably by protein to protein interaction. The same conclusions can be drawn from dark field observation of these grids (Fig. 6c). When similar grids were rotary shadowed, the DNA appeared uniform and well contrasted. On the contrary, the HU–DNA complex appeared diffused and more irregular with many loops and hairpin structure (Fig. 6d and e). Contrary to DNA unwinding proteins (25), no destabilization of the double helix is observed. The last point to be underlined is that all the λ-Eco RI DNA fragments bind to HU protein.

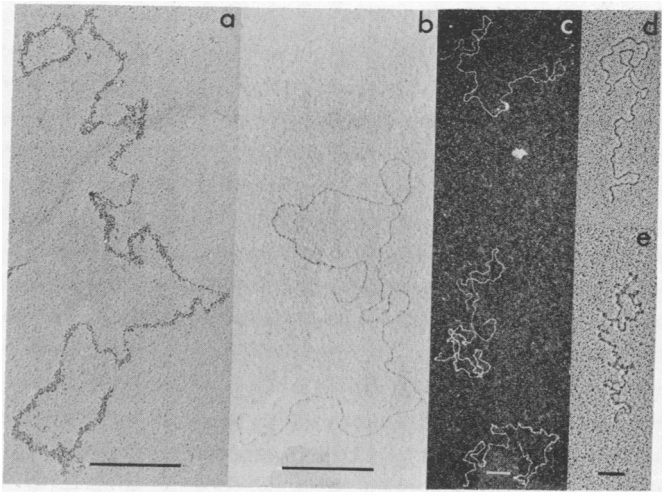

FIG. 6. Electron microscopy studies. Eco RI-cleaved λ DNA was mixed with an equal weight of HU in 0.4 M KCl; 10 mM Tris·HCl (pH 7.5); 0.1 mM EDTA. The ionic strength was progressively reduced to 0.06 M KCl in the same buffer. Carbon-coated copper grids were activated in amylamine vapor according to Dubochet *et al.* (24). The samples of DNA or DNA + HU were diluted in 10 mM Tris·HCl (pH 7.5), 1 mM EDTA, applied to the grids, and stained with an 0.5% aqueous uranyl acetate for 10 sec. The DNA or DNA–protein complexes were visualized in different manners. Bright field observation with a Philips 301: (a) DNA + HU; (b) DNA alone. Dark field observation with a Philips 301: (c) mixture of DNA–HU complex and free DNA. The top two molecules are naked DNA; the bottom two are DNA–HU complexes. Rotary shadowing with Pt-Pd observed with a Siemens Elmishop 101: (d) DNA alone; (e) DNA + HU. The bar indicates 100 nm.

DISCUSSION

By affinity chromatography on a DNA-cellulose column we have purified a small, heat-stable polypeptide with a molecular weight of 7,000 that stimulates *in vitro* transcription of λ DNA. This protein is relatively abundant in *E. coli* cells; a minimal estimate of 10,000 molecules per cell was made. In the absence of DNA, the native form is probably a dimer; further experiments are necessary to establish the oligomeric form of the protein when associated with DNA.

The association between HU and λ DNA in a stoichiometric ratio largely increases the initial rate of transcription by *E. coli* RNA polymerase. Recent experiments showed that *E. coli* H_1 factor preferentially affects the expression of certain types of promoters (3). By contrast, we could not detect by hybridization to separated strands of λ DNA and to λ*imm* 21 DNA any specific stimulation by HU of certain types of promoters on λ DNA. It is possible that HU increases the efficiency of all the promoters of λ DNA to a similar extent. In fact, electron microscopy studies indicate that it binds to the six Eco RI fragments of λ DNA. These studies also suggest that HU binds along the DNA double helical chain, causing an apparent thickening of the DNA. Loops and hairpins are formed, suggesting a more condensed structure, a result in agreement with the HU-dependent increase in the sedimentation velocity of λ DNA. It is interesting to recall that, on the basis of electron microscopy studies on normal or Pronase-digested extruded content of osmotically shocked *Salmonella typhimurium* cells, Hamkalo and Miller (26) have postulated that bacterial DNA is associated with proteins uniformly along its length. However, the number of HU copies, 10,000 per cell (although it may be largely underestimated), is far below the number of HU molecules necessary to cover the bacterial chromosome according to the stoichiometry deduced from *in vitro* experiments. This discrepancy can be explained either by the presence of other protein(s) with a similar role or by the existence of limited number of preferred sites along the DNA molecule.

Another indication of the tight association between HU and DNA is the stabilization of A+T-rich regions in double-stranded λ DNA, as recorded by differential melting experiments (Cl. Reiss and J. Yaniv, manuscript in preparation).

These properties of HU are reminiscent of the association of eukaryotic histones with double-stranded DNA. In fact, when the amino acid composition of HU is compared with that of histones H2B and H1 (22, 23), certain similarities are observed. HU may contain a structured hydrophobic region and a nonstructured region rich in lysine and alanine. Firmer conclusion on the relationship between HU and eukaryotic histones will have to await the results of amino-acid sequence analysis and physicochemical studies.

Recent experiments (J. Yaniv, unpublished results) show that HU protein can be separated into two fractions by phosphocellulose chromatography. These two fractions are identical in their mobility on neutral or denaturing gels as well as in their amino-acid composition after acid hydrolysis. Furthermore, no difference was observed between these two subfractions by immunodiffusion or immunoelectrophoresis. These subfractions could represent two forms of the HU protein.

Note Added in Proof. A DNA-binding protein, immunologically related to HU, has been isolated from several strains of blue-green algae. It seems likely that HU has been strongly conserved in the prokaryotes (R. Haselkorn and J. Rouvière-Yaniv, manuscript in preparation).

We are grateful to M. Yaniv for advice and encouragement throughout this work, and to G. Cohen, P. Truffa-Bacchi, P. Kourilsky, H. Buc, G. Pieltre, and J. Merlie for helpful discussions. We thank G. Le Bras and M. Gouyvenaux for performing the amino acid analysis and M. Perricaudet for performing the suberimidate experiment. We are indebted to C. Brack, O. Croissant, and J. Dubochet for help and advice in electron microscopy studies. This work was supported by grants from the Centre National de la Recherche Scientifique and the Commissariat à l'Energie Atomique.

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