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Interaction between the *Chlamydia trachomatis* Histone H1-Like Protein (Hc1) and DNA

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The gene encoding the *Chlamydia trachomatis* histone H1-like protein (Hc1) from serovar L2 was cloned into *Escherichia coli* by use of expression vector pET11d. In this vector, transcription of the gene is under the control of a bacteriophage T7 promoter, and T7 RNA polymerase is inducible in the host. Following induction, the *E. coli* cells were lysed gently. Gel filtration of the lysate revealed comigration of DNA and Hc1 in the voided volume. Electron microscopy revealed the DNA to be complexed with protein in large aggregates, often in the form of spherical bodies. Purified recombinant Hc1 maintained its DNA-binding capacity and was able at high concentrations to form condensed aggregates with DNA (one molecule of Hc1 per base pair) independently of the form or size of the DNA but with a slight preference for supercoiled DNA. Hc1 alone is thus able to package DNA into condensed spherical bodies.

Chlamydia trachomatis is an obligate intracellular bacterium with a unique biphasic life cycle in which the extracellular elementary bodies (EBs) alternate with the intracellular reticulate bodies (RBs). EBs are small (0.3 μm in diameter), infectious, but metabolically inactive. They are surrounded by an outer membrane, which is heavily cross-linked by disulfide bonds. EBs adhere to susceptible host cells and induce their own phagocytosis. At 4 to 6 h postinfection, EBs are transformed to RBs, which are larger (1 μm in diameter), noninfectious, but metabolically active. They divide by binary fission. During their intracellular phase, RBs remain within the phagosome, in which they form the inclusion. At 24 to 48 h postinfection, the inclusion is filled with RBs, the transition from RBs to EBs takes place, and the inclusion bursts, liberating infectious EBs. During the transition from RBs to EBs, the diameter of the particle is reduced by a factor of three, causing a 30-fold volume reduction. In RBs, as in other bacteria, the DNA is loosely packed, occupying a major part of the cytosol. A dramatic reorganization of the chlamydial chromosome is therefore required for accommodation of its DNA in the much smaller EBs. Electron microscopy of thin-sectioned chlamydiae revealed that in EBs, the nucleoid is seen as a dark, dense body positioned in the center of the cell (9), indicating condensation of the DNA.

Proteins responsible for such condensation should be synthesized late in the developmental life cycle. Wagar and Stephens (34) reported the detection of two cytoplasmic DNA-binding proteins synthesized late in the developmental life cycle. These proteins were able to bind both double- and single-stranded DNAs. These authors suggested that these proteins could be involved in the condensation of the chlamydial chromosome during the transition from RBs to EBs.

Hackstadt et al. (17) identified a developmentally regulated *C. trachomatis* protein with high homology to eucaryotic histone H1. The protein was expressed only during the late stages of the chlamydial life cycle. The gene encoding this protein was cloned into *Escherichia coli*, in which it was shown to cause condensation of the *E. coli* nucleoid (2). Fractionation of lysed *E. coli* on sucrose gradients revealed cosedimentation of DNA and the recombinant *C. trachomatis* histone H1-like protein (Hc1).

In this paper, we describe the interaction of recombinant Hc1 and DNA. Recombinant Hc1 was shown to bind nick-translated double-stranded DNA by Southwestern (DNA-protein) blotting. Bound DNA could be released when the filters were washed in buffers containing 500 mM sodium chloride. After gentle lysis of recombinant *E. coli*, gel filtration revealed comigration of Hc1 and DNA, and electron microscopy of fractions containing both components revealed DNA-protein aggregates often condensed into compact spherical bodies. Structurally similar bodies were formed when purified Hc1 was mixed with DNA at a concentration of one molecule of Hc1 per base pair of DNA. These DNA-protein complexes were formed spontaneously, even at low (1 to 20 μg of DNA per ml) concentrations of DNA.

MATERIALS AND METHODS

Cultivation of *C. trachomatis*. *C. trachomatis* serovar L2 LGV-II/434/Bu was cultivated in monolayers of McCoy cells (obtained from the American Type Culture Collection, Rockville, Md.) and purified (4). The purified EBs were used to prepare protein lysates and DNA.

Measurement of Hc1 and DNA concentrations in EBs. The Hc1/DNA weight ratio in EBs was determined by use of purified EBs suspended in phosphate-buffered saline (PBS) containing 1.3 mM each $MgCl_2$ and $CaCl_2$. Extracellular DNA and RNA were digested with DNase I (10 μg/ml) and RNase A (20 μg/ml) (both from Worthington Biochemical Corp., Freehold, N.J.) for 45 min at 37°C. EDTA was then added to a 2 mM final concentration, and EBs were purified by density gradient centrifugation as described previously (4). Purified EBs were washed once with distilled water to

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lyse any RBs and pelleted. The EB pellet was resuspended in PBS before analysis of protein and DNA contents.

For measurement of the DNA content, the EB suspension was digested with proteinase K (0.6 mg/ml; Boehringer, Mannheim, Germany) for 30 min at 37°C and diluted 1:1,000 in TNE buffer (10 mM Tris [pH 7.4], 1 mM EDTA, 100 mM NaCl) containing 0.1 μg of Hoechst 33258 (Sigma Chemical Co., St. Louis, Mo.) per ml. The DNA concentration was determined by fluorescence measurements with a TKO minifluorometer (Hoefer Scientific Instruments, San Francisco, Calif.). pBluescript KS+ plasmid DNA (Stratagene, La Jolla, Calif.) was used for calibration.

The protein concentrations of the EB suspension and of purified Hc1 were estimated by Bradford analysis with protein assay reagents (Bio-Rad Laboratories, Richmond, Calif.) according to the manufacturer's instructions. For determination of the amount of Hc1 in EBs, samples of known protein concentration were mixed with sodium dodecyl sulfate (SDS) sample buffer (0.125 mM Tris [pH 6.8], 2.4% [wt/vol] SDS, 10% [wt/vol] glycerol, 5% [vol/vol] bromphenol blue solution) and boiled for 5 min before separation on SDS–14% polyacrylamide gels. Gels were stained with Coomassie blue and dried before being scanned on a Shimadzu dual-wavelength thin-layer chromatography scanner (CS-930) connected to a Shimadzu data recorder (DR-2).

Bacterial strains and vectors. *E. coli* XL1-Blue host cells were used for the initial electrotransformation. The *E. coli* host lysogen BL21 (DE3)/pLysS (32) was used for Hc1 expression with vector pET11d (Novagen, Madison, Wis.).

Preparation of rabbit antibody. One New Zealand White rabbit was immunized with purified EBs (K23) or with purified Hc1 (K101) essentially as described by Birkelund and Andersen (3). At days 1 and 4, 20 μg of antigen suspended in equal volumes of PBS and Freund complete adjuvant (Difco Laboratories, Detroit, Mich.) was given intramuscularly. The same amount of antigen suspended in Freund incomplete adjuvant (Difco) was injected as described above at days 8, 11, 15, and 18. At days 49 and 56, 50 μg of antigen in PBS was given in the marginal vein of the ear. At day 70, the rabbit was bled, and the serum was stored in 5-ml portions at −70°C.

Cloning of *hc1* in pET11d. Synthetic oligonucleotides containing 5′-terminal sequences for *Nco*I and *Bam*HI restriction endonucleases were prepared by use of an automated oligonucleotide synthesizer (380B; Applied Biosystems, Inc., Foster City, Calif.). The 5′ oligonucleotide contained the nucleotide sequence coding for the amino-terminal region of the L2 *hc1* gene (16) and an *Nco*I restriction endonuclease site (5′-GGA TAT TTT TCC ATG GCG CTA AAA GAT ACG GC-3′). The 3′ oligonucleotide contained the sequence located 45 nucleotides downstream from the carboxy terminus of the *hc1* gene and a *Bam*HI restriction endonuclease site (5′-GGG GGA TCC TCA ATG AGA AGG GGA AAG AGG GCC C-3′).

The *hc1* gene was amplified for 30 cycles in a Perkin-Elmer Cetus thermal cycler from pCtX3-61, which contains the *hc1* gene (6), by use of the above-described synthetic oligonucleotides and *Thermus aquaticus* polymerase. The amplified *hc1* gene product was ligated into pET11d after digestion with *Nco*I and *Bam*HI and transformed into *E. coli* XL1-Blue host cells. Transformation was performed by electroporation with a Gene Pulser apparatus (Bio-Rad). A preparation of pET11d with *hc1* from the transformed XL1-Blue cells was sequenced as a control and used to electrotransform BL21(DE3)/pLysS host cells. The resulting transformant clone, *E. coli* BL21(DE3)/pLysS/pET11d*hc1* (recombinant Hc1), was used in all subsequent experiments.

Cultivation and induction of recombinant bacteria. Recombinant Hc1 bacteria were grown at 37°C in 500 ml of LB medium containing ampicillin (0.1 mg/ml) and chloramphenicol (0.017 mg/ml) as described by Sambrook et al. (28).

When cells had reached an optical density at 600 nm of 0.3, protein production was induced by the addition of 1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) and glucose (2.8 g/liter). At 30 min postinduction, rifampin (0.2 mg/ml) was added, and cells were incubated for a further 2 h. Finally, cells were harvested by centrifugation (2,500 × *g* for 15 min at 4°C). The pellet was kept on ice prior to lysis and purification of Hc1.

For testing for induction, 1-ml culture samples were collected before and after induction, centrifuged down at 10,000 × *g* for 5 min, solubilized in 100 μl of SDS sample buffer without β-mercaptoethanol, and boiled for 3 min.

Isolation of Hc1-DNA complexes. For isolation of protein-DNA complexes, recombinant Hc1 bacteria were grown as described above, except that glucose was omitted. At 1.5 h after the addition of rifampin, a 5-ml culture sample was harvested by centrifugation (5,000 × *g* for 15 min at 4°C), and pelleted cells were subjected to gentle lysis by the addition of 300 μl of ice-cold lysis buffer (150 mM NaCl, 20 mM Tris [pH 7.5], 1 mM EDTA, 0.05% [vol/vol] Tween 20) for 5 min at 25°C. RNA and cellular debris were removed by centrifugation (20,000 × *g* for 10 min) after treatment with RNase A (13 μg/ml) for 5 min at 25°C.

The supernatant (200 μl) was filtered through a Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden) gel filtration column with PBS (pH 7.4) as the washing buffer. Collected fractions (200 μl) were used immediately for protein and DNA analyses.

DNA-binding assay. Proteins from induced recombinant Hc1 bacteria were solubilized in SDS sample buffer and separated on SDS–14% polyacrylamide gels (22) with a bisacrylamide/acrylamide ratio of 1:40. Western (immunoblot) transfer was done by the Bio-Rad transfer procedure with BA85 nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany) (4). The membranes were blocked for 15 min at 37°C in PBS (pH 7.4) containing 5% (wt/vol) bovine serum albumin and 0.02% (wt/vol) sodium azide.

The DNA-binding assay was performed as described by Wagar and Stephens (34). Blocked nitrocellulose membranes were washed three times (15 min each time) in PBS containing 0.05% (vol/vol) Tween 20. The membranes were incubated in 100 ml of PBS-Tween 20 with nick-translated genomic chlamydial DNA labelled with [^{32}P]dATP (10^8 cpm; Amersham, Amersham, Buckinghamshire, England) for 1 h at room temperature with gentle agitation (the L2 DNA was extracted as described by McClenaghan et al. [23] and nick translated by standard procedures [28]). After incubation, the membranes were washed three times (30 min each time) in PBS-Tween 20 and one time (15 min) in PBS without Tween 20. After the washes, the membranes were dried and exposed to RX-100 films (Fuji).

SDS-PAGE and immunoblotting. Proteins in SDS sample buffer were separated on polyacrylamide gels by polyacrylamide gel electrophoresis (PAGE) and transferred to nitrocellulose membranes (22). Nitrocellulose sheets containing electrophoretically transferred antigens were blocked for 15 min in 20 mM Tris (pH 7.5)–150 mM NaCl–3% (wt/vol) gelatin (5). Polyclonal rabbit antiserum (150 μl) raised against L2 or purified Hc1 was absorbed with 600 μl of *E. coli* lysate (Bio-Rad) for 15 min at room temperature, diluted

1:40 with antibody buffer (20 mM Tris [pH 7.5], 150 mM NaCl, 0.2% [wt/vol] gelatin, 0.05% [wt/vol] Tween 20), and incubated with the membranes for 1 h at 37°C. Binding of the antibodies was detected with goat anti-rabbit immunoglobulin G conjugated to alkaline phosphatase (Bio-Rad), and staining was done with BCIP/NTB color development solution (Bio-Rad) in accordance with the instructions given by the manufacturer.

Electrophoretic transfer of Hc1-DNA complexes separated on agarose gels. Fractionated protein-DNA complexes were separated on 0.7% NA-agarose (Pharmacia) gels immediately after gel filtration. Twenty microliters of each fraction was applied to the gels and electrophoresed in Tris-borate buffer (TBE) containing 90 mM Tris, 90 mM borate, 2 mM EDTA (pH 7.8), and ethidium bromide (0.5 mg/liter). After electrophoresis, the gels were soaked in TBE and placed between two sheets of nitrocellulose. Electrophoretic transfer was carried out for 1 h at 4°C in TBE. The membranes were blocked in gelatin and reacted with rabbit hyperimmune serum as described for immunoblotting.

Agarose gel electrophoresis and Southern blotting. Plasmid DNA or DNA-Hc1 complexes were separated on 0.7% agarose gels by electrophoresis and Southern transferred by standard procedures (28).

Purification of Hc1 protein. Protein was extracted from overproducing cells essentially as described by Harlow and Lane (18). The pelleted bacteria were suspended in 12.5 ml of an ice-cold solution consisting of NaCl (100 mM), EDTA (1 mM), Tris (50 mM, pH 8), and sodium deoxycholate (0.1% [wt/vol]). Lysis was allowed to proceed for 20 min at 37°C, and then NaCl was added to 0.5 M and $MgCl_2$ and $CaCl_2$ were each added to 5 mM. DNase I (4 μg/ml) was added to the mixture, which was then incubated for 30 min at 37°C. The mixture was cleared by centrifugation at 20,000 × *g* for 10 min. Ammonium sulfate equal to 80% saturation was added to the supernatant, which was then incubated for 30 min at room temperature. The precipitate was collected by centrifugation at 10,000 × *g* for 10 min.

Purification was carried out at 4°C with the programmer GP-250 Plus and a FRAC-100 fraction collector (Pharmacia LKB Biotechnology, Uppsala, Sweden). The ammonium sulfate pellet was dissolved in 20 ml of CE buffer (10 mM $NaCO_3$ [pH 10], 1 mM EDTA) (17) containing 3% glycerol (27) and loaded onto a 7-ml heparin-Sepharose CL6B (Pharmacia) column at a flow rate of 0.5 ml/min. Following adsorption to the column and a wash, the target protein was eluted with a linear salt gradient from 0 to 1 M NaCl in CE buffer and 1-ml fractions were collected in glass tubes containing 200 μl of Tris-HCl (1 M, pH 8). Fractions were stored in 50% glycerol (vol/vol) at −20°C until use. Concentration of the protein was determined by Bradford analysis with Bio-Rad protein assay reagents.

In vitro formation of Hc1-DNA complexes. Purified pBluescript KS+ DNA of 3.0 kbp and at concentrations of 1, 2, 5, 10, and 20 μg/ml was mixed with dilutions of purified Hc1 protein (100, 50, 20, 10, 4, and 2 μg/ml). The samples were incubated for 5 min at 37°C prior to analysis.

Detection of Hc1 by ELISA. For the enzyme-linked immunosorbent assay (ELISA), 0.6 μg of pBluescript KS+ DNA was mixed with 6 μg of Hc1 in PBS, and the mixture was incubated for 5 min at 37°C. DNA-Hc1 complexes were separated by gel filtration on a Sepharose 4B column. The fractions (50 μl) were applied to polystyrene microtiter plates (F-16; Nunc, Roskilde, Denmark) and incubated for 1 h at 37°C. The wells were blocked with 3% gelatin in PBS, and Hc1 was detected with K101 serum diluted 1:200 in PBS.

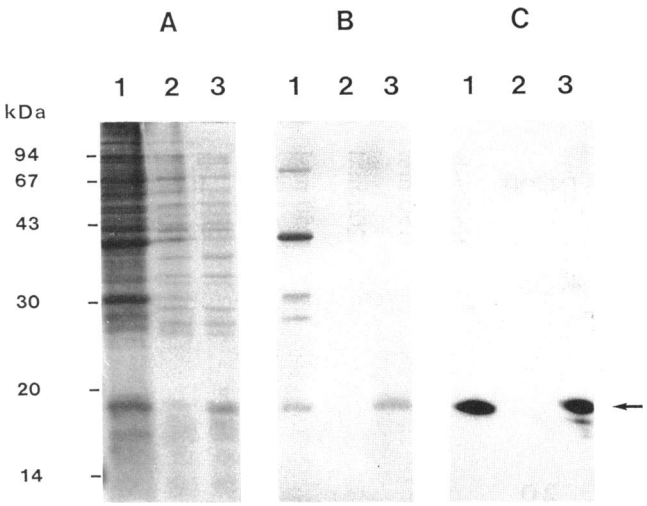

FIG. 1. (A) SDS–14% polyacrylamide gel stained with Coomassie blue. Lanes: 1, *C. trachomatis* L2 proteins; 2, uninduced recombinant Hc1 *E. coli* proteins; 3, recombinant Hc1 *E. coli* proteins induced for 2 h. (B) Immunoblotting of an SDS-polyacrylamide gel run in parallel with the gel in panel A. Hyperimmune rabbit serum against *C. trachomatis* L2 (K23) was used for the reaction. (C) Southwestern blotting of an SDS-polyacrylamide gel run in parallel with the gel in panel A and reacted with nick-translated *C. trachomatis* L2 double-stranded DNA. The arrow indicates the position of the 18-kDa polypeptide. No reaction with uninduced recombinant Hc1 cultures was seen (B and C).

Rabbit immunoglobulin G was detected with an anti-rabbit immunoglobulin G–horseradish peroxidase conjugate (Bio-Rad) diluted 1:3,000 in PBS and the substrate 1,2-phenylenediamine dihydrochloride (DAKO, Glostrup, Denmark).

Electron microscopy of Hc1-DNA complexes. Ten microliters of fractionated protein-DNA complexes or reconstituted Hc1-DNA complexes was applied to carbon-coated, glow-discharged 400-mesh copper grids (Polaron; Johnson, Charlottenlund, Denmark) for 5 min. The grids were transferred to a spermidine-containing buffer (2 mM spermidine, 150 mM NaCl) (19), rinsed sequentially in 25, 50, 75, and 95% ethanol, air dried, and rotary shadowed with tungsten (15).

RESULTS

Hc1 expression. The gene encoding the Hc1 protein from *C. trachomatis* L2 was cloned into *E. coli* BL21(DE3)/pLysS by use of expression vector pET11d (32). This system allows the expression of otherwise toxic proteins (20) because a double *lac* repressor construction prevents any transcription of the lysogenized T7 polymerase gene on the host chromosome and of the vector insert downstream of the T7 promoter. The *lacI* gene is also present on both the host chromosome and the vector to provide sufficient repression. Transcription is therefore prevented until induction with IPTG. During induction, T7 lysozyme is induced in small amounts along with Hc1 because of pLysS. The expression of recombinant Hc1 protein in *E. coli* is shown in Fig. 1. Protein profiles (Fig. 1A) of cultures of recombinant Hc1 without induction (lane 2) and after 2 h of induction with IPTG (lane 3) were compared with those of *C. trachomatis* L2 (lane 1). A protein band migrating like the *C. trachomatis* L2 Hc1 protein band can be seen in lane 3 (arrow). Hyperimmune rabbit serum against chlamydia EBs (K23) reacted with the 18-kDa polypeptides of *C. trachomatis* L2 and

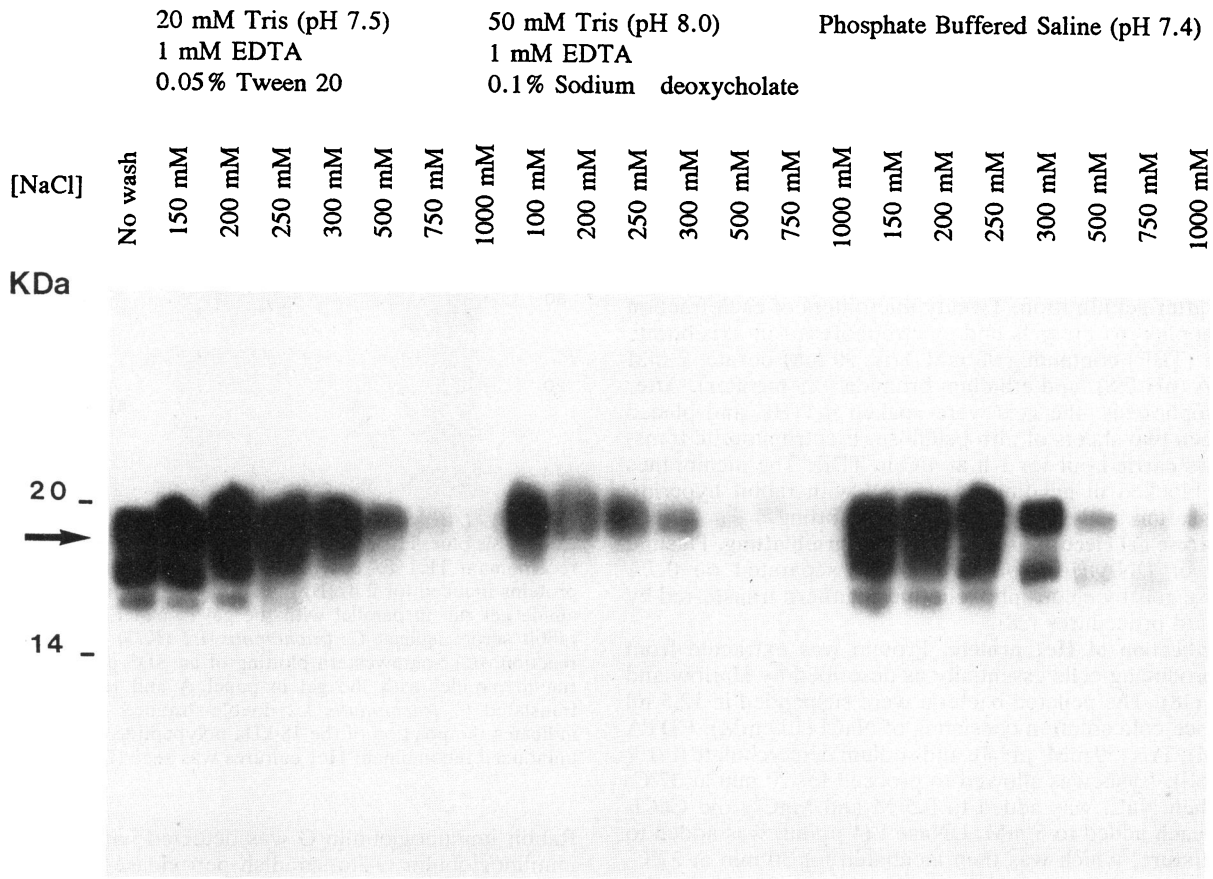

FIG. 2. Influence of the sodium chloride concentration on DNA binding by Hc1. Hc1 production was induced for 2 h in recombinant Hc1 *E. coli*. The proteins were separated by SDS–14% PAGE and electrotransferred to a nitrocellulose membrane. The membrane was incubated with ^{32}P-labelled *C. trachomatis* L2 DNA. After a wash in PBS-Tween 20, the membrane was cut into strips. The strips were washed with increasing concentrations of NaCl in one of the three buffers listed at the top of the figure. The arrow indicates the binding of labelled DNA to Hc1.

induced *E. coli*, whereas no reactivity with uninduced cells was observed (Fig. 1B). Southwestern blotting of the gel (Fig. 1C) revealed the binding of DNA to the 18-kDa band. Prolonged induction resulted in no additional production of Hc1, probably because of its toxicity for the *E. coli* cells.

Binding of recombinant Hc1 to DNA. As shown in Fig. 1C, DNA binds to recombinant Hc1. To determine the conditions under which such Hc1-DNA complexes could be isolated, we analyzed the stability of the Hc1-DNA interaction. Recombinant Hc1 bacteria were induced with IPTG for 2 h. Cells were pelleted by centrifugation and lysed, and the proteins were solubilized in SDS sample buffer and separated on polyacrylamide gels by electrophoresis. The proteins were transferred to nitrocellulose membranes, which were then incubated with nick-translated double stranded *C. trachomatis* L2 DNA. For testing for the stability of the DNA-Hc1 interaction, the membranes were sliced and subjected to washings in different buffers containing increasing concentrations of sodium chloride. As shown in Fig. 2, washing in buffers containing more than 500 mM NaCl released the labelled DNA. The buffer containing 20 mM Tris, 1 mM EDTA, and 0.05% Tween 20 stabilized the DNA-protein complexes, whereas the buffer containing sodium deoxycholate released most of the radioactive DNA, even at 100 mM NaCl. A sharp release of radioactivity was seen with 500 mM NaCl in PBS. Divalent cations did not influence the binding of DNA to Hc1.

Isolation of DNA-protein complexes formed in vivo. The induction of Hc1 is toxic for *E. coli* (17). This toxicity could be caused by a DNA-protein interaction. To analyze the DNA-protein interaction, we isolated DNA-protein complexes from gently lysed recombinant Hc1 cell cultures 2 h after induction. PBS containing 0.05% Tween 20 was used for lysis. Following lysis, the sample was filtered through a Sepharose 4B column, and the fractions were analyzed by gel electrophoresis. Agarose gel electrophoresis (Fig. 3A) revealed the presence of DNA in fractions 5 to 7 (voided volume). Only a small fraction of the DNA entered the gel, while most of the DNA remained in the slots. Electrotransfer of the agarose gel to a nitrocellulose filter revealed that Hc1 detected by hyperimmune serum K23 was eluted in the same fractions and comigrated with DNA (Fig. 3B). Hc1 migrated to the anode despite its predicted positive net charge at the pH of the transfer buffer, indicating the interaction to be charge related. Proteins from each fraction were separated by SDS-PAGE and transferred to a nitrocellulose membrane. An 18-kDa DNA-binding protein was eluted in fractions 5 to 7, which also contained DNA. For analysis of the DNA that failed to enter the agarose gel, samples from fractions 5 to 8 of the gel filtration column were subjected to

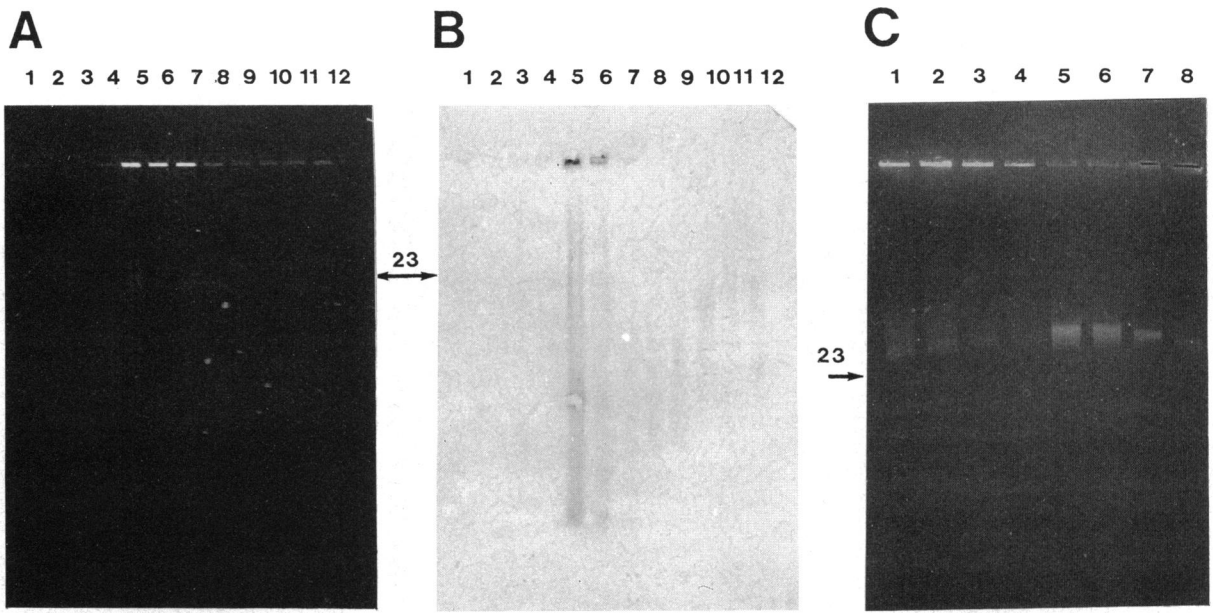

FIG. 3. Recombinant Hc1 *E. coli* was induced for 2 h and lysed in lysis buffer, and the supernatant was fractionated on a Sepharose 4B column. Twelve fractions were collected. (A) Ethidium bromide-stained 0.7% agarose gel of fractions 1 to 12; fraction numbers are indicated over the lanes. (B) Electrotransfer of the agarose gel to a nitrocellulose filter. For immunodetection, rabbit antiserum (K23) was used. Fraction numbers are indicated over the blot. (C) Ethidium bromide-stained 0.7% agarose gel. Lanes 1 to 4 show fractions 5 to 8 from panel A; lanes 5 to 8 show the same fractions extracted with phenol-chloroform. The arrows indicate the 23-kb DNA fragment.

phenol-chloroform extraction. As shown in Fig. 3C, most of the DNA that remained in the slots was fragmented chromosomal DNA of about 23 kbp. Southern hybridization with an end-labelled T7 primer revealed, however, that plasmid DNA also was retained in the slots, indicating a protein-DNA interaction. From uninduced cultures, lysed in the same buffer after the addition of lysozyme, very little DNA, detectable only by electron microscopy, was released.

Purification of recombinant Hc1 protein. Recombinant Hc1 protein was purified from overproducing recombinant Hc1 *E. coli* bacteria. After lysis in buffer containing sodium deoxycholate, the protein was concentrated by precipitation with 80% ammonium sulfate. The protein was then purified on a column of heparin-Sepharose CL6B (16), which binds Hc1. The purified protein was eluted from the column with 650 to 870 mM NaCl at a concentration of 200 μg/ml, reacted with K23 antiserum, and bound radioactive double-stranded DNA. The purified recombinant protein was thus similar to the purified *C. trachomatis* L2 protein described by Hackstadt (16).

In vitro reconstitution of Hc1-DNA complexes. DNA-Hc1 complexes formed in vivo and in vitro were compared. Hc1-DNA complexes reconstituted in vitro were obtained by mixing diluted solutions of pBluescript KS+ plasmid DNA with serial dilutions of purified recombinant Hc1 protein. Complexes were formed in PBS after 5 min of incubation at 37°C and analyzed on agarose gels (Fig. 4). At high concentrations of Hc1 protein (1 μg of Hc1 per 0.2 μg of DNA), the plasmid DNA was trapped at the top of the gel. At 0.5 and 0.2 μg of Hc1, some of the plasmid DNA migrated into the gel. At lower concentrations, the DNA migrated somewhat more slowly than DNA not subjected to Hc1 complex formation. There was a slight preference for form I DNA to be retained in the complexes at 0.5 and 0.2 μg of Hc1 protein.

Fractionation of Hc1-DNA complexes formed in vitro. Hc1-DNA complexes formed between 6 μg of Hc1 and 0.6 μg of DNA were fractionated on a Sepharose 4B column, and the fractions were analyzed for the presence of Hc1 by an ELISA. Hc1 was eluted in fractions 5 to 8, similar to the elution of complexes formed in vivo. Purified Hc1 protein separated on a similar column was eluted in fractions 10 and

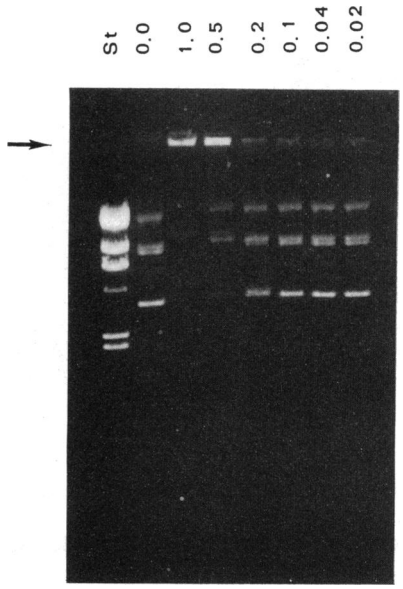

FIG. 4. Gel retardation assays with 0.7% agarose gels. pBluescript KS+ DNA (0.2 μg) was mixed with purified Hc1 for 5 min. The amounts of Hc1 in micrograms are listed over the lanes. Lane St is *Hin*dIII-cleaved lambda DNA. The arrow indicates the position of the slots for DNA application.

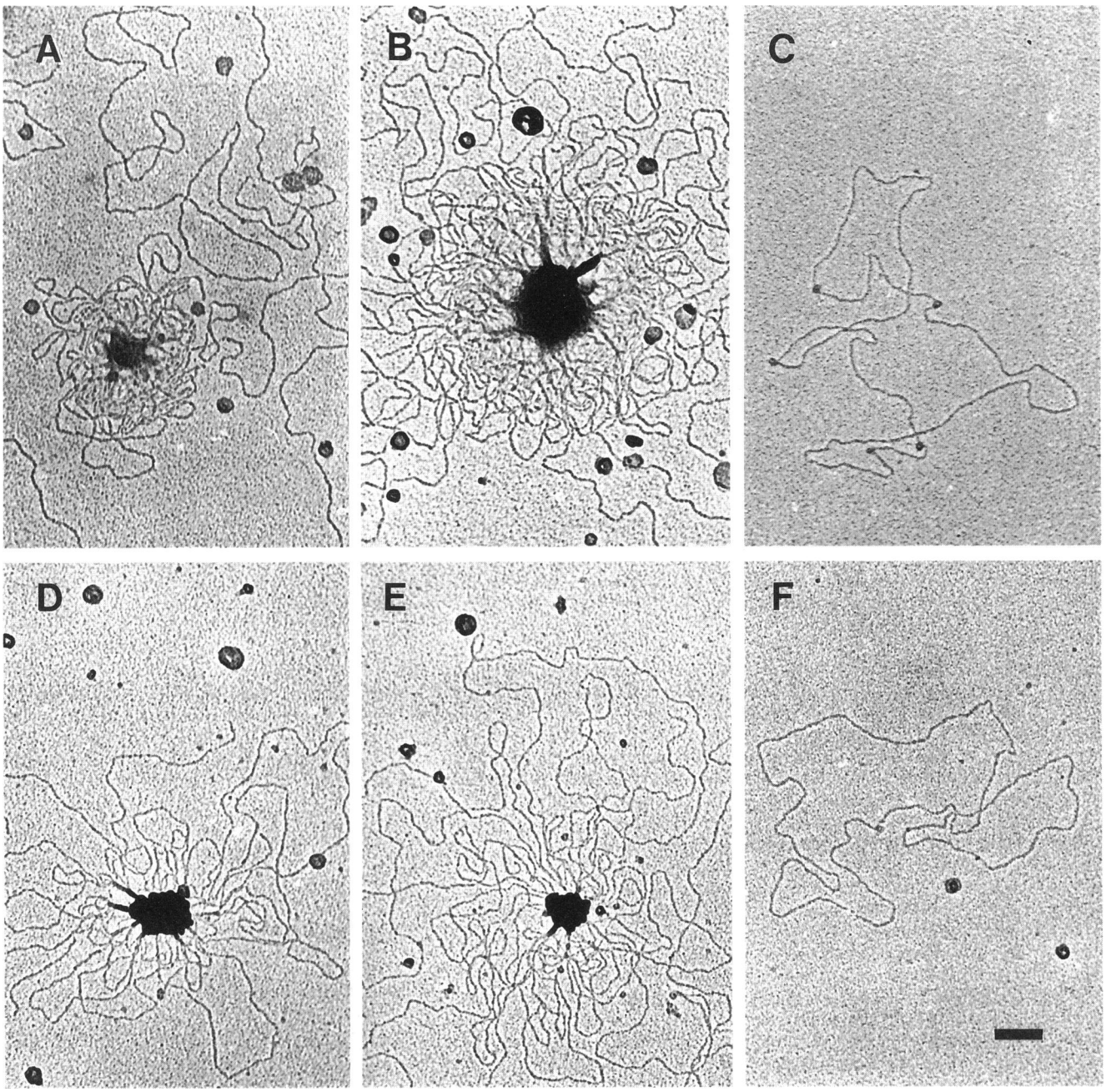

FIG. 5. Electron microscopy of protein-DNA complexes from recombinant Hc1 *E. coli* as analyzed by the direct mounting technique. Cells were lysed in lysis buffer, RNase treated, and subsequently separated on Sepharose 4B columns. DNA-containing fractions were subjected to electron microscopy. (A and B) DNA-protein complexes from cells induced for 2 h. (C) DNA-protein complexes from uninduced cells. (D and E) Glutaraldehyde-fixed DNA-protein complexes from induced cells. (F) Glutaraldehyde-fixed DNA-protein complexes from uninduced cells. Bar, 0.1 μm.

11, indicating that only DNA-bound protein was eluted in the voided volume.

Electron microscopy of Hc1-DNA complexes. Complexes formed in *E. coli* between Hc1 and DNA were analyzed by electron microscopy by use of the direct mounting procedure described by Griffith and Christiansen (15). Following lysis and gel filtration, fractions containing DNA (Fig. 3A) were mounted for electron microscopy. Examples of complexes are shown in Fig. 5. Around an electron-dense center loops of DNA can be seen. Close to the center, the DNA forms a tight meshwork, in which it is impossible to follow individual DNA strands. In the periphery, loops and linear fragments of well-spread DNA can be seen. The sizes of the complexes vary from small to very large (Fig. 5A and B). Fixation in 0.8% glutaraldehyde prior to mounting and dehydration did not change the appearance of the complexes (Fig. 5D and E). From samples of uninduced cells prepared in parallel with the induced cells, much less material was released. The

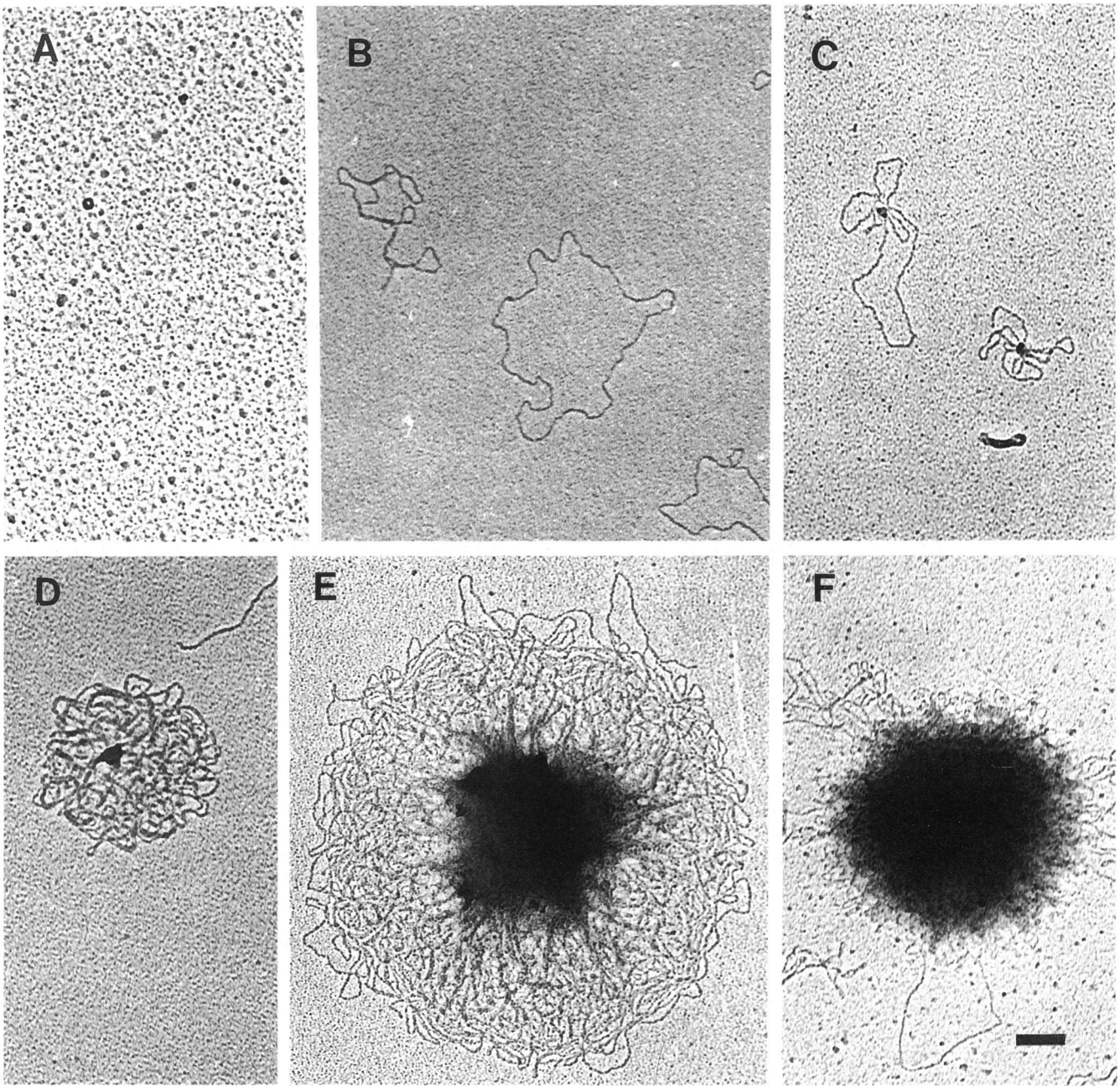

FIG. 6. Electron micrographs of complexes formed in vitro between purified Hc1 and pBluescript KS+ DNA (10 μg/ml). (A) Purified recombinant Hc1 (100 μg/ml). (B) pBluescript KS+ DNA. (C to F) Hc1-DNA complexes: C, 1 Hc1 molecule per 88 nucleotides (1:88); D, 1:44; E, 1:11; F, 1:2.2. Bar, 0.1 μm.

amount of DNA was too low to be detected by agarose gel electrophoresis. By electron microscopy, no DNA-protein complexes were detected, and only plasmid DNA was released (Fig. 5C and F). Chromosomal DNA was retained within the bacterial cells.

Complexes formed in vitro were prepared at molar ratios of 1 Hc1 molecule per 88 nucleotides to 1 Hc1 molecule per 1 nucleotide. Uncomplexed Hc1 at a concentration of 100 μg/ml is shown in Fig. 6A. There is no self-association of Hc1 in the absence of DNA. Uncomplexed pBluescript KS+ DNA is shown in Fig. 6B. Most of the DNA is supercoiled, but open circles are also present in the DNA preparation. For complex formation, a DNA concentration of 10 μg/ml was used. Complexes formed at ratios of 1 Hc1 per 88 nucleotides (Fig. 6C), 1 Hc1 per 44 nucleotides (Fig. 6D), 1 Hc1 per 11 nucleotides (Fig. 6E), and 1 Hc1 per 2.2 nucleotides (Fig. 6F). At lower ratios of protein to DNA, no complex formation was observed by electron microscopy. At an Hc1/nucleotide ratio of 1:88, complexes between Hc1 and DNA started to form. Around a small electron-dense center, the DNA formed loops and coils. One or a few plasmid molecules participated in complex formation (Fig.

6C). At an Hc1/nucleotide ratio of 1:44, larger complexes formed. The dark center became larger and was surrounded by tight loops and coils of DNA fibers. Several plasmid molecules participated in complex formation (Fig. 6D). Hc1-DNA complexes formed at a ratio of 1:11 were much larger. From the condensed center, thicker fibers protruded and were surrounded by a corona of DNA loops (Fig. 6E). At the highest ratio, 1:2.2, an almost total condensation of the DNA-protein complex occurred. The condensed center occupied most of the complex. Towards the periphery of the complex, there was a less dense region in which tightly wound DNA loops were seen. Not all plasmid molecules participated in complex formation (Fig. 6F). The coexistence of free DNA and Hc1-DNA complexes showed that the binding of Hc1 to DNA was cooperative. Complex formation was independent of the DNA concentration. Complexes formed at an Hc1 concentration of 1, 5, or 10 μg/ml were indistinguishable in appearance and size, indicating cooperative binding of Hc1 to DNA.

For analysis of complex formation between Hc1 and linear DNA, pBluescript KS+ DNA was cleaved with *Hin*dIII, which linearizes the plasmid (Fig. 7A). Complexes were formed at Hc1/nucleotide molar ratios of 1:44, 1:22, 1:5.5, and 1:1.1 when a DNA concentration of 5 μg/ml was used. Complexes very similar to the complexes formed with supercoiled DNA were observed (Fig. 7B to E).

When pBluescript KS+ DNA was cleaved with *Hae*III, 14 fragments from 605 to 11 bp were produced (Fig. 7F). Complexes were formed between Hc1 and DNA at a DNA concentration of 5 μg/ml. No complexes were observed at a Hc1/nucleotide ratio of 1:44. At a ratio of 1:22, a few large complexes were observed (Fig. 7G). The complexes were similar to the complexes formed with linear DNA; however, numerous free ends of linear DNA were observed at the periphery of the complexes. At a ratio of 1:5.5 (Fig. 7H), the complexes were about the same size as those in Fig. 7G; the DNA, however, was more kinked. This kinkiness was also observed with the free DNA molecules. At the highest ratio, 1:1.1, the structure of the complexes was quite different from the structure formed with long linear or supercoiled molecules at this ratio. The small linear fragments were not condensed to a dense structure but consisted of a dense center surrounded by a corona of heavily kinked short DNA fragments (Fig. 7I).

In vitro complexes of Hc1 and DNA can thus be formed at low concentrations of DNA (5 to 10 μg/ml) independently of the form of DNA but highly dependent on the Hc1 concentration. We used concentrations of Hc1 from 1 to 50 μg/ml in the complex reactions. Complex formation was observed at 2 to 50 μg of Hc1 per ml. The molar ratios at which complex formation occurred were 1:88 (Fig. 6C) to 1:1.1 (Fig. 6E and I).

These ratios were compared to the in vivo ratios in EBs. We measured the relative amount of Hc1 by scanning a Coomassie blue-stained gel. Scanning of a gel with nonreduced proteins showed that Hc1 was equal to 6% of the total protein, compared with 5.4% when proteins were reduced. The ratio of total protein to DNA was determined to be 10.08, yielding 0.6 μg of Hc1 per μg of DNA or 1 molecule of Hc1 per 37 nucleotides. This value is thus within the range of those for in vitro complex formation.

Given the size of the *C. trachomatis* chromosome (6), the total number of Hc1 molecules in one EB is 26,000. The concentration of Hc1 within an EB (0.3 μm in diameter) is 46 mg/ml. This high concentration should facilitate complex formation in vivo.

DISCUSSION

Compared with that of the well-characterized eucaryotic chromatin, the structure of the procaryotic chromatin is remarkably unknown. The major reason is that the procaryotic chromatin is much less stable when subjected to purification and analysis. The procaryotic chromosome is compacted into a nucleoid of the bacterial cell. Compaction is required to accommodate the chromosome in the cell; however, the mode of compaction is not yet fully determined (21). Electron micrographs of a gently released nucleoid showed that beaded loops of protein-DNA complexes surrounded the lysed bacterial body (14). Upon slightly longer incubation or lysis in 1 M NaCl (10), supercoiled DNA loops replaced the fragile beaded fibers. It has so far not been possible to isolate and characterize the beaded procaryotic chromosome fibers. In *E. coli*, there are several histone-like proteins, the most abundant being the HU protein, which shares some properties with eucaryotic histones. However, it has not been shown to interact as a unit with DNA to form complexes analogous to eucaryotic nucleosomes (11).

The unique biphasic life cycle of chlamydiae necessitates a dramatic reduction in the volume of the cell when RBs are reorganized to EBs late in the chlamydial life cycle. In RBs, the chromatin structure is similar to the structure seen in other bacteria, whereas in EBs, the chromatin packed into a condensed nucleoid unique to procaryotes (9). Two proteins with DNA-binding capability are synthesized late in the developmental life cycle (34). The genes encoding two late proteins similar in size to the proteins described by Wagar and Stephens (34) have been cloned and sequenced by Hackstadt et al. (17) and by Perara et al. (25). Both of these late proteins are very basic and show apparent homology to eucaryotic histone H1. The 18-kDa protein described by Hackstadt et al. (17) is conserved in *C. trachomatis*. The protein described by Perara et al. (25) varies in size from 32 to 23 kDa, depending on the serovar (16). Both proteins are abundant in EBs. Even though the genes encoding these late DNA-binding chlamydial proteins have been sequenced, their possible role in the inactivation and condensation of the nucleoid remains to be determined. In the study by Barry et al. (2), recombinant *E. coli* expressing Hc1 was capable of condensing *E. coli* nucleoids, as visualized by acridine orange staining. Purified nucleoids were isolated, and Hc1 was shown to cosediment with the nucleoid particles by sedimentation in sucrose gradients. In this study, we showed that purified Hc1 at high concentrations can bind and aggregate double-stranded DNA. At high ratios of protein to DNA, the complexes remained in the slots of the agarose gel. Supercoiled DNA was preferentially retarded; however, open circles and linear fragments also participated in complex formation. Since Hc1 could form complexes with a large number of DNA molecules, as seen by electron microscopy, we did not attempt to characterize the retardation further. Electron microscopy of the complexes showed that large aggregates were formed at high ratios of Hc1 to DNA. At no ratio could nucleosome-like structures or compaction be detected. At low ratios, complexes involving a single DNA molecule were formed. These complexes were formed around a condensation center, in which coiling of the DNA could be seen. DNA not participating in the coiling remained unchanged. It thus seems that Hc1 can induce loop formation in the DNA. The lowest ratio at which complex formation could be detected was 1 monomer of Hc1 per 88 nucleotides. The structure of the small complexes indicated that numerous Hc1 molecules must participate in complex

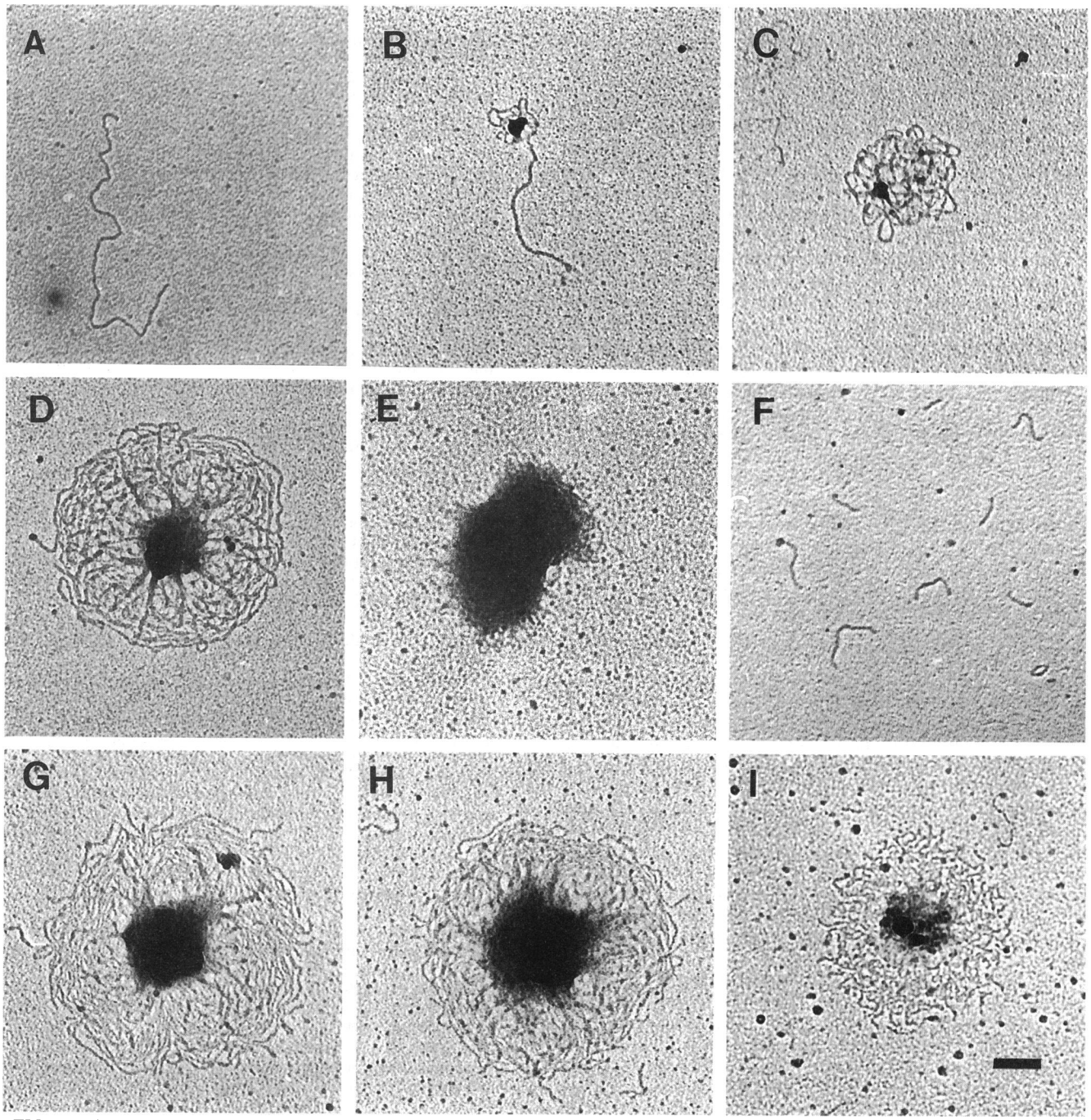

FIG. 7. Electron micrographs of complexes formed in vitro between purified Hc1 and restriction endonuclease-cleaved pBluescript KS+ DNA (5 μg/ml). (A to E) DNA cleaved with *Hin*dIII. (F to I) DNA cleaved with *Hae*III. (A and F) pBluescript KS+ DNA. (B to E and G to I) Complexes formed at a ratio of 1 Hc1 molecule per 44 nucleotides (1:44) (B), at 1:22 (C and G), at 1:5.5 (D and H), and at 1:1.1 (E and I). Bar, 0.1 μm.

formation, probably through a protein-protein interaction, and that the binding of Hc1 to DNA is cooperative. This conclusion is also indicated by the fact that complex formation was largely independent of DNA concentration but highly dependent on protein concentration.

Other microorganisms contain DNA-binding proteins that functionally are related to Hc1. In *Bacillus subtilis*, abundant (10 to 20% of total spore protein) small, acid-soluble proteins of the α/β type are synthesized within the developing spore late in sporulation and degraded early in spore germination (31). They are associated with spore DNA and alter DNA conformation from the B to the A form (24). They confer UV resistance to the DNA (13) and cause DNA condensation in recombinant *E. coli* similar to the condensation of spore DNA (30).

In thermophilic archaebacteria, thermal denaturation of

the bacterial chromosome must be prevented by factors such as a high intracellular salt concentration or DNA-binding proteins. A DNA-binding protein with homology to eucaryotic core histones was described for *Methanothermus fervidus* (29). This protein binds to double-stranded DNA, increases resistance to thermal denaturation, and results in the formation of quasispherical DNA-protein complexes that resemble nucleosomes. It is thus likely that DNA-binding proteins have evolved to fulfill specific needs for the protection and condensation of DNA in different bacteria.

Hc1 was found by a data base search to be similar to the C-terminal end of eucaryotic histone H1. Lysine-rich histone H1 is the most variable of the histones (8). Histone H1 is less conserved than the core histones that constitute the nucleosomes. It is essential for the folding of chromatin into solenoids (36) and can act as a suppressor for gene activity caused by tissue-specific packaging (35).

Histone H1 sequences exhibit a three-domain structure (12), the central domain of which is a folded, globular domain responsible for the binding of histone H1 at its specific localization on the chromatin fiber. The N-terminal domain is probably responsible for the functional variation, while the C-terminal domain most likely is responsible for the condensation of DNA (1). Histone H1 can bind to DNA, forming rod-shaped, sometimes circular structures 11 to 15 nm in diameter and 1.6 times shorter than DNA. They contain 70% (wt/wt) histone H1-DNA and several DNA molecules (7). The C-terminal domain of histone H1 can form similar structures with DNA (26), while the central globular domain can form bridges between two DNA strands (33). Even though these structures are different from the structures described in the present paper, the tendency of the C-terminal domain to form toroidal particles and the ability of the globular domain to bind DNA side by side may be reflected in the structures formed by Hc1 and DNA.

The DNA-Hc1 complexes released from lysed *E. coli* are very similar to the complexes formed in vitro. It was surprising that there was no sign of beading as a result of an interaction with the *E. coli* chromatin fiber. It was also surprising that the complexes formed in vivo consisted mainly of chromosomal DNA and not of plasmid DNA. It is possible that the production of Hc1 in *E. coli* can damage the cells and thereby change the structure of bacterial chromatin.

The demonstration of large complexes formed between Hc1 and DNA in vitro indicates that a similar process could be involved in the packaging of chlamydial DNA during the transition from RBs to EBs, when the amount of Hc1 is abundant (2, 34). The structures formed between Hc1 and DNA are unique but show some similarities to the structures formed by eucaryotic histone H1 and DNA. The structure of chlamydial chromatin, the position of Hc1, and its possible interaction with the 26-kDa DNA-binding protein remain to be determined. The preferential binding of Hc1 to supercoiled DNA may reflect a possible role in gene inactivation as well.

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