Purification and Characterization of a DNA-Binding Protein of *Vibrio cholerae* O1

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Abstract: A DNA−Binding protein was purified from the cell-surface of *Vibrio cholerae* O1 by Cellulofine Sulfate affinity chromatography and reversed-phase HPLC. The DNA−binding protein of *V. cholerae* O1 showed a molecular weight of 9 kDa estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Treatment of the protein with cold acetone and 6 M guanidine-HCl produced little change in the DNA−binding activity of the protein. The binding of the protein to supercoiled plasmid DNA as well as to double-stranded short chromosomal DNA fragments has been demonstrated by gel retardation assay and by labeling of the transblotted protein with non-radioactive DNA. *N*−terminal amino acid sequence of the protein showed a high degree of homology with that of HU 2 (Laine *et al.*, 1978), NS2 (Mende *et al.*, 1978) from *Escherichia coli.* Indirect immunocytochemical labeling of ultrathin sections of Lowicryl−embedded *V. cholerae* O1 cells with the antibody against the protein revealed that the protein associates with bulk DNA and also lies at the cell-surface.

Key words: DNA−binding protein, *Vibrio cholerae* O1

INTRODUCTION

In bacteria, several proteins with DNA−binding properties have been implicated in folding and condensing the long circular DNA molecule, although the mechanism responsible for DNA packaging in prokaryotes is less clear than that operating in eukaryotic cells. Among these proteins, HU (NS), functionally similar to histones, is the most abundant (estimated at 2×10^4 to 1×10^5 molecules per cell) and best characterized (Drlica and Rouviere-Yaniv, 1987; Pettijohn, 1988). HU protein is a heat stable, small basic protein which wraps DNA into nucleosome like structures (Rouviere-Yaniv *et al.*, 1979; Broyles and Pettijohn, 1986). It exhibits several important functions *in vitro.* It is involved in DNA replication starting at *E. coli oriC* (Dixon and Kornberg, 1984), in phage Mu transposition (Craigie *et al.*,

Received for Publication, September 13, 1991.
Contribution No. 2537 from the Institute of Tropical Medicine, Nagasaki University.

1985), and in site-specific DNA inversion (Johnson *et al.*, 1986; Wada *et al.*, 1988). HU protein is composed of two closely related 10 kDa monomer subunits that form stable heterodimers in solution (Rouviere-Yaniv and Kjeldgaad, 1979; Bonnefoy *et al.*, 1989). The *hup A* and *hup B* genes encoding the HU−α and HU−β subunits are located at 90.5 and 9.7 min, respectively, on the *E. coli* map (Wada *et al.*, 1988). Double mutants lacking HU protein were constructed, and cultures of *hup A hup B* mutant bacteria were found to contain filaments, anucleate cells, however, the isolated HU mutants were viable under normal growth condition (Wada *et al.*, 1988; Dri *et al.*, 1991).

Recently, we have purified a DNA−binding protein from the cell−surface of *V. cholerae* O1 which is termed here HVc **H**istone like protein of ***Vibrio cholerae*** O1). This paper describes the purification, characterization and localization of the HVc protein. We also compared the protein concentration of HVc among vibrio strains with special reference to pathogenicity and lysogenicity.

Materials and Methods

Bacterial strains. The bacterial strains used in this study are listed in Table 1.

Preparation of shear-fraction. Vibrio cells were grown on TCG agar (Ehara *et al.*, 1987) in 100 Roux bottles containing 100 ml of medium at 30℃ for 48 h. After the addition of 10 ml of Tris-buffered saline (TBS1, 20 mM Tris−HCl, pH 8.0, 0.15 M NaCl) to each bottle, cells were harvested by pipette and centrifuged at 10,000×*g* for 1 h at 4℃. Whole cells were treated with cold acetone (−20℃) for 20 min on ice and centrifuged at 10,000×*g* for 15 min at 4℃. The cell pellet was dried on filter paper at −20℃ (acetone powder). The acetone powder was resuspended in TBS1 to 0.5 g (dry weight) /ml, then homogenized with a cooled Sorvall Omnimixer at setting 6 for 5 min. The homogenate was centrifuged at 12,000×*g* for 30 min at 4℃. The resultant supernatant was pooled at 4℃ and the pellet was resuspended with fresh TBS1 and the homogenization repeated three times. Each supernatant was combined and kept at 4℃ overnight after the addition of DNase I and RNase, each at 100 μg/ml. The mixture was centrifuged at 12,000×*g* for 1 h at 4℃ and the supernatant was designated as shear-fraction.

Table 1. Bacterial strains

Strains	Biotype	Prophage−typing	Source
34/D13	Classical		El Tor station, 1930 by Dr. Doorenbos
K23−7	El Tor	Celebes original	Kenya, 1984
Chiba 3	El Tor	cured	Japan, 1978
Ubon 13	El Tor	Ubol	Ubon, 1960 by Dr. Felsenfeld
U 38	El Tor	Ubol	Ubon, 1964 by Dr. Finkelstein, R.A.

Cellulofine Sulfate affinity column chromatography. Cellulofine Sulfate (CS resin) is a spherical cellulose bead functionalized with a low concentration of sulfate esters (SEIKAGAKU KOGYO, Co. Ltd., Japan). CS resin was washed with deionized water (10 times-volume of gel bed) and equilibrated with TBS1 at 4℃. After loading the shear-fraction, the column was washed with TBS1 until the O.D. of the eluate at 280 nm was below 0.005. Elution was started with TBS1 containing 1 M NaCl. Each eluted fraction was monitored by SDS−PAGE and electron microscopy (EM).

Purification of DNA−binding protein. Purification of DNA−binding proteins were performed in two strains of K23−7 and Chiba 3.

Method 1: Purification from strain K23−7. CS resin eluted fractions were combined and dialyzed against 0.1 M morpholinoethane sulfonic acid (MES), pH 6.8 containing 1 mM guanosine−5'−triphosphate (GTP), 1 mM ehyleneglycol−*O, O'*−bis (2−amino ethyl)−*N,N,N',N'*−tetraacetic acid (EGTA) and 0.5 mM $MgCl_2$ at 4℃ overnight. The dialysate was centrifuged at 100,000×*g* for 1 h at 4℃. The supernatant was dialyzed against deionized water, vacuum-evaporated and loaded onto a reversed-phase HPLC after treatment with 6 M guanidine−HCl. Additionally, the pellet was resuspended in 1 ml of deionized water and dialyzed against deionized water. The dialysate was vacuum-evaporated and further fractionated by a reversed-phase HPLC. The presence if 9 kDa protein in eluted fractions suggested that the separation of 9 kDa protein from other proteins was still incomplete, therefore we combined those fractions containing 9 kDa protein into two portions and vacuum-evaporated. Each portion was further incubated in MES buffer in the presence of 1 mM adenosine−5'−triphosphate (ATP), 1 mM GTP, 0.5 mM $MgCl_2$, 1 mM EGTA and 20 μM Taxol for 30 min at 37℃ in an attempt to separate 9 kDa protein from other acidic proteins. Taxol was distributed by the Drug Synthesis & Chemistry Branch, Division of Cancer Treatment, National Cancer Institute, Executive Plaza North, Suite 831, Bethesda, Maryland 20892, U.S.A. These mixtures were centrifuged at 100,000×*g* for 1 h at 30℃, then monitored by SDS−PAGE and EM.

Method 2: Purification from strain Chiba 3. Fractions eluted from CS resin affinity chromatography were combined and dialyzed against MES buffer containing 1 mM ATP, 1 mM GTP, 0.5 mM $MgCl_2$ and 1 mM EGTA at 4℃ overnight. The dialysate was centrifuged at 100,000×*g* for 1 h at 4℃. The pellet and the supernatant were monitored by a SDS−PAGE. The supernatant was further fractionated by a reversed-phase HPLC.

Reversed-phase HPLC. Samples for HPLC were dialyzed against deionized water and lyophilized. The lyophilized samples were dissolved in 1 ml of 6 M guanidine−HCl solution, then incubated at 37℃ for 1 h and injected. Column conditions were as follows: for purification from strain K23−7, column; μ BONDASPHERE 5 μ C8−300A, 19 mm×15 cm (Waters, division of MILLIPORE), flow rate; 4 ml/min, fraction; 4 ml/tube, eluent A; H_2O/0.05% trifluoroacetic acid (TFA), eluent B; CH_3CN/0.05% TFA, gradient; 0−80% B, 60 min, linear gradient, detection; UV 220 nm, and for purification from Chiba 3, column; μ BONDASPHERE 5 μ C18−300A, 3.9 mm×15 cm, flow rate; 1 ml/min, fraction; 1 ml/tube, the other conditions were same with those used for K23−7.

DNA preparation. Chromosomal DNA was purified from strain K23−7 by lysis with 1% SDS and proteinase K (1 mg/ml) (MERCK) in 20 mM phosphate-buffered saline, pH 7.2 and phenol-chloroform-ether extraction methods of Maniatis *et al.* (1982). Ethanol-precipitated DNA was washed once with 70% ethanol. Purified DNA was double-digested with *Hind* III and *Eco*RI, then labeled with digoxigenin-conjugated dUTP (Boehringer Mannheim), as specified by the manufacturer. The plasmid pBR 322 DNA was purchased from Wako Pharmaceuticals, Co. Ltd..

DNA−binding protein assays. Assay for DNA−binding to electroblotted proteins was performed by using modifications to previously published procedures (Dooley *et al.*, 1988; Wager and Stephens, 1988). All steps were performed at room temperature. Nitrocellulose membranes with blotted proteins were blocked for 30 min at 37℃ with Block Ace (DAINIPPON Pharmaceutical, Co. Ltd.). The blots were then washed twice for 10 min in 10 mM Tris−HCl−buffered saline, pH 7.4 (TBS2) containing 0.05% Tween 20 (Wako) (TBS2−T) and washed once for 10 min in TBS2. Washed blots were probed for 2 h with 0.1 μg of labeled DNA per ml in TBS or incubated in TBS2 alone. Probed blots were blocked again for 30 min at 37℃ with Block Ace and washed twice for 10 min in TBS2. Protein−DNA complexes were detected by an anti-digoxigenin antibody conjugated to alkaline phosphatase (Boehringer Mannheim) as specified by the manufacturer. In addition to the method described as above, using gel electrophoresis the band-retardation technique was applied to obtain further evidence for the DNA affinity of the 9 kDa protein from *V. cholerae* O1 (samples used were from the fraction 36 in Fig. 3). Plasmid pBR 322 DNA samples (0.5 μg in 4 μl) containing different concentrations of the 9 kDa protein were incubated for 15 min at 37℃ in a final volume of 6 μl of the binding buffer {50 mM NaCl, 20 mM Tris−HCl, pH 8.0, 1 mM ethylenediaminetetraacetic acid (EDTA)}. Prior to loading on the gel, a cold stop solution containing 0.5 M EDTA, pH 8.0, 50% glycerol and bromophenol blue was added.

Preparation of antisera to HVc proteins purified from strains of K23−7 and Chiba 3. HPLC−purified HVc proteins from both strains were treated with 1% SDS and mixed with equal volume of Freund's complete adjuvant (1 ml of HVc protein solution of 50 μg/ml and 1 ml of the adjuvant) respectively. Rabbits were injected subcutaneously at two sites in the back and intramuscularly at two sites in the thigh every two weeks (5 times) substituting incomplete adjuvant for complete adjuvant. They were bled one week after the last injection.

Western blot analysis of HVc proteins among strains of *V. cholerae* O1 with different prophage-typing. Strains as listed in Table 1 were cultured on TCG agar plates, harvested and suspended in 20 mM Tris−HCl, pH 8.0, containing 0.4 M NaCl and broken in a French press with a pressure of 17,000 p.s.i. The salt concentration of 0.4 M was used to separate HVc protein from DNA according to the method of Rouviere-Yaniv and Gros (1975). Crude extracts were centrifuged at 12,000×*g* for 30 min at 4℃ to remove the cell debris. The protein concentration of each supernatant was adjusted equally with the same buffer used in cell-suspension. HVc proteins were searched by western blotting, using the monospecific antiserum against the HVc protein purified from strain K23−7. Out of these

five strains, only the pathogenic strain K23−7 produces the kappa phage and the strain 34/D 13 is non-pathogenic and a non-producer of any phages.

SDS−PAGE. SDS−PAGE was performed in 1.5 mm thick slab gels according to the system of Laemmli (1970). Samples were applied to the polyacrylamide slab gel (15%) using a 5% stacking gel and were electrophoresed for 7 h at 25 mA constant current per slab. The protein bands were stained with Coomassie brilliant blue R250. For molecular weight calibration, a low molecular weight marker kit (Pharmacia LKB) was used.

Agarose gel electrophoresis. Agarose gel electrophoresis was performed as described in Maniatis *et al.* (1982), applying 0.04 M Tris-acetate buffer, pH 8.0. Positions of DNA bands were stained by ethidium bromide.

Western blotting. Immunoblotting was performed according to the techniques of Towbin *et al.* (1979). Crude and purified DNA binding protein preparations were subjected to SDS−PAGE in duplicate on different gels, one of which was stained with Coomassie blue and the other electroblotted onto nitrocellulose membrane (Bio−Rad) using a Bio−Rad electroblotting apparatus (30 V for 16 h and 70 V for 1h). Horseradish-peroxidase-conjugated goat anti-rabbit IgG was from Cappel Laboratories Inc., U.S.A. and 4−chloro−1−naphtol used in the development of the color reaction was from Bio-Rad Laboratories.

Electron microscopy (EM). For negative staining, one drop of the sample was placed on a sheet of Parafilm and a Formvar-coated copper grid was floated on the drop for 2 min. The excess liquid was removed with filter paper. The specimen was washed three times with distilled water each time for 10 s, then stained with 1% uranyl acetate for 30 s. The excess stain was removed, using the edge of a piece of filter paper. For immunoelectron microscopy, vibrio cells (strain K23−7) cultured on TCG agar for 48 h at 30℃ were fixed with 2% paraformaldehyde in PEM buffer {5 mM piperazine−*N,N'*−bis (2−ethanesulfonic acid), 2 mM EGTA, 5 mM $MgCl_2$, pH 6.1 adjusted with 1 N NaOH} for 1 h at room temperature. Then, specimens were dehydrated by an ethanol gradient at −30℃ and embedded in Lowicryl K4M resin. Polymerization of K4M was done at −30℃ under UV−light as recommended by the manufacturer. Following polymerization, ultrathin sections were prepared on an ultramicrotome (OmU2, C. Reichert Optische Werke AG, Vienna, Austria), and the sections were mounted on nickel grids. These grids were incubated on drops (30 μl) of the diluted antiserum (1:500, anti−K23−7 HVc antiserum) for 15 min. After incubation, grids were washed 3 times successively with drops of deionized water. The excess fluid was removed with the edge of filter paper, and grids were further incubated with goat anti-rabbit IgG antiserum coated with 15 nm-colloidal gold (Janssen) for 15 min. Grids were washed 3 times with deionized water, air-dried, and stained with uranyl acetate and lead citrate. Specimens were examined with a JEM 100CX electron microscope operated at 80 kV.

N−terminal amino acid sequence of HVc proteins. The sample was run on a protein sequencer system of Applied Biosystem, ABI 477 A/120 A according to the method as recommended by the manufacturer.

RESULTS

CS resin affinity chromatography. The proteins were eluted in one large peak from fractions 8 to 20 and the SDS−PAGE profile is shown (Fig. 1). A similar elution profile was obtained for the strain Chiba 3 preparation (data not shown).

Purification of a DNA−binding protein.

Method 1: Purification from strain K23−7. The vacuum-evaporated sample was loaded onto a reversed-phase HPLC (C8) after treatment with 6 M guanidine−HCl. The elution profile is shown in Fig. 2. As the fractionation of the proteins was not satisfactory by this HPLC mthod, we modified the method for protein separation. Taking a small aliquot of fraction No. 33, we incubated the sample with 0.1 M MES and 4 M glycerol as mentioned in "MATERIALS AND METHODS." We found that a 55 kDa protein disapperaed with this treatment. The original fraction No. 33 contained the 55 kDa protein, although only fraction No. 33 treated as described above is shown in Fig. 2. Fraction Nos. 31 to 43 were combined, vacuum-evaporated and dissolved in the above MES buffer, followed by a 30 min-incubation at 37℃. After the incubation, the sample was centrifuged at 100,000×*g* for 1 h at 30℃. The resultant supernatant was dialyzed against deionized water for 2 days at 4℃, then vacuum-evaporated. The evaporated sample was loaded again onto the reversed phase HPLC after the treatment with 6 M guanidine−HCl. The elution profile is shown in Fig. 3. A small aliquot of fraction No. 36 was loaded onto a protein sequencer. The pellet from the fraction ob-

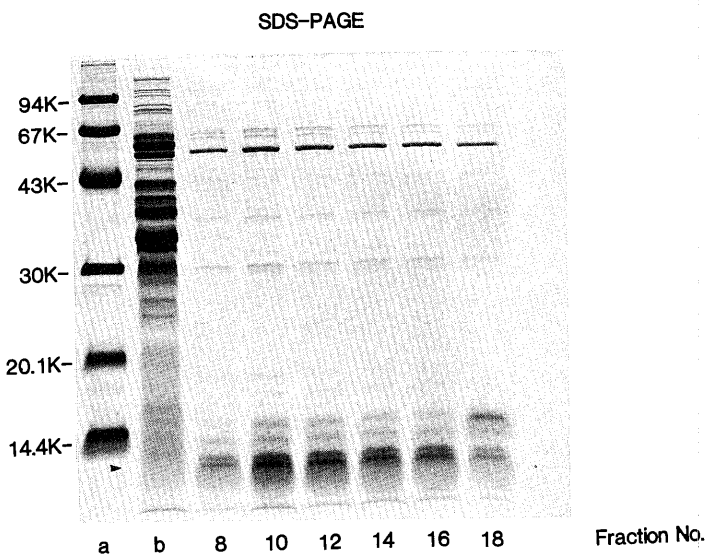

Fig. 1. SDS−PAGE analysis of the shear-fraction eluted from Cellulofine Sulfate affinity chromatography. Lanes: a; low molecular weight marker proteins (LMMPs), b; sample loaded, 8 to 18; fraction Nos. Sizes are given in kilodaltons. An arrowhead indicates the 9 kDa protein. Note that the 9 kDa protein is shown as a doublet band.

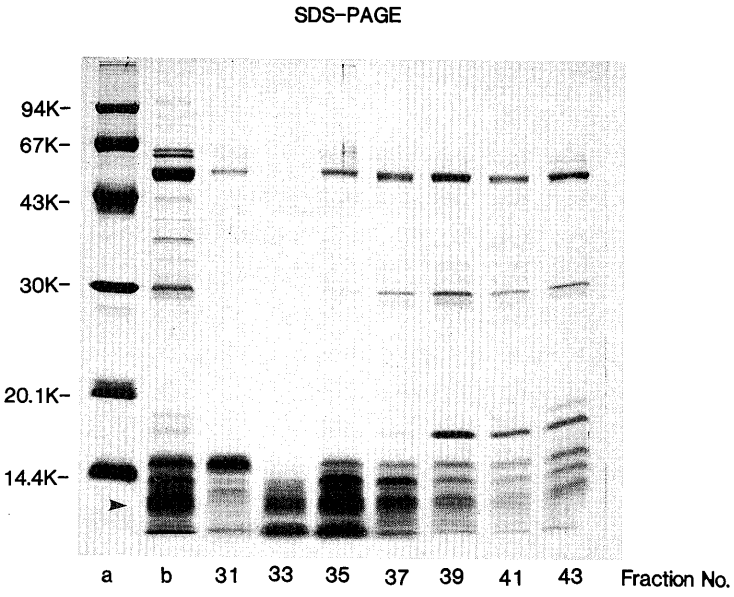

Fig. 2. SDS−PAGE analysis of the fractions eluted by reversed-phase HPLC. Lanes: a; LMMPs, b; CS resin-eluted sample, 31 to 43; fraction Nos. used in HPLC. Each fraction eluted by HPLC were vacuum-evaporated and resuspended in deionized water except No. 33. Fraction No. 33 was resuspended in MES buffer as described in MATERIALS AND METHODS. Note that fraction No. 33 lacks the 55 kDa protein band.

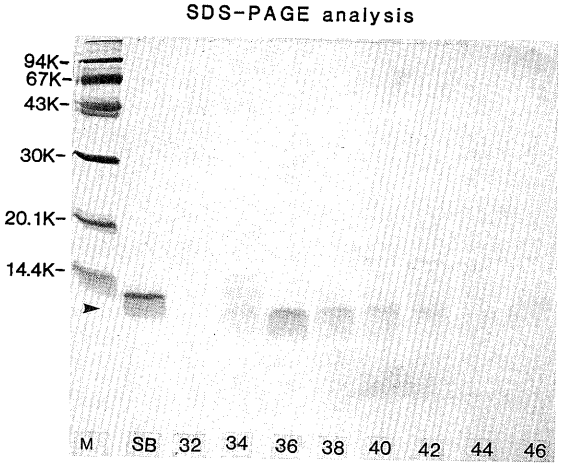

Fig. 3. SDS−PAGE profile of the fractions re-chromatographed by reversed-phase HPLC. Fraction Nos. 31 to 43 (Fig. 2) were combined and vacuum-evaporated. Sample loaded: $100{,}000 \times g - 1$ h−supernatant of the shear-fraction treated as mentioned in RESULTS. Lanes: M; LMMPs, SB; cholera toxin subunit B used as a molecular weight marker of 10 kDa, 32 to 46; fraction Nos. applied to reversed-phase HPLC. An arrow-head indicates the 9 kDa protein shown as a single band in fraction No. 36.

tained by 100,000×g−1 h−centrifugation was resuspended in 1 ml of deionized water, then dialyzed against deionized water for 2 days at 4℃. The dialysate was vacuum-evaporated and fractionated by reversed-phase HPLC after treatment with 6 M guanidine−HCl. The elution profile is shown in Fig. 4. As shown in this profile, the 9 kDa protein still remained in the fractions No. 31 to No. 41. Referring to the presence of 16 kDa protein, these fractions were combined to two portions (Nos. 29 to 34 and Nos. 35 to 41). The combined fractions were vacuum-evaporated and dissolved in a modified MES buffer (0.1 M MES, pH 6.8 1 mM

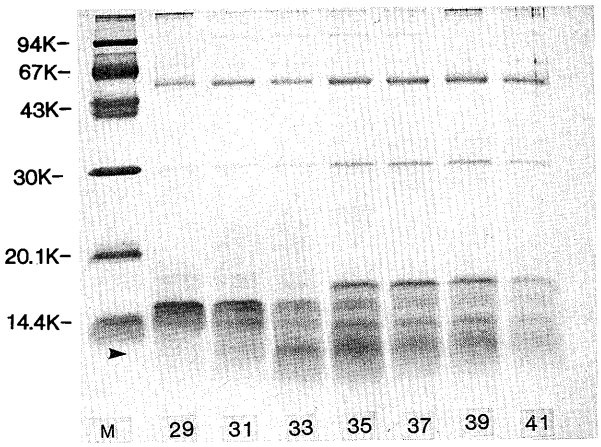

Fig. 4. SDS−PAGE analysis of 100,000×g−1 h−pellet of the shear-fraction treated as mentioned in RESULTS. Lanes: M; LMMPs, 29 to 41; fraction Nos. of a reversed phase HPLC. Note that the 9 kDa protein still remaines as indicated by an arrowhead.

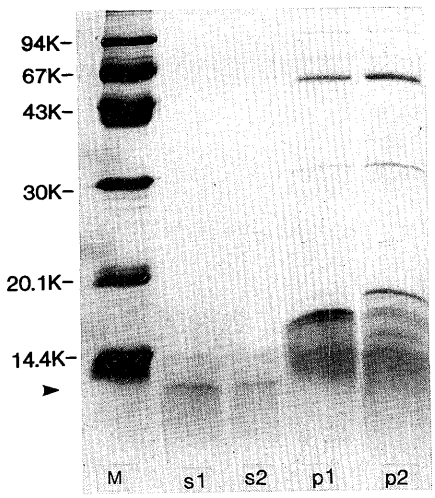

Fig. 5. Effect of ATP and GTP on the release of the 9 kDa protein from an unidentified filamentous complex. Lanes: M; LMMPs, sl and s2; 100,000×g−1 h−supernatants of the combined fractions (29 to 34 and 35 to 41) treated as mentioned in RESULTS, p1 and p2; pellets of each sample. Note that the 9 kDa protein band is seen only in s1 and s2.

EGTA, 0.5 mM $MgCl_2$, 1 mM GTP, 1 mM ATP and 20 μM Taxol), followed by a 30 min−incubation at 37℃. Both samples were centrifuged at 100,000×g for 1 h at 30℃. The results of SDS−PAGE of the supernate and the pellet are shown in Fig. 5. Note that the 9 kDa protein bands are seen only in lanes sl, s2 and not in pl, p2. An EM picture of fraction p2 is shown in Fig. 6. Samples taken from sl were used for one of the DNA−binding protein assays.

Method 2: Purification from strain Chiba 3. In the previous method, we found that the presence of 1 mM ATP and GTP was effective to separate the 9 kDa protein from the others (see Figs. 3 and 5). Therefore, fractions eluted from CS resin affinity chromatography were dialyzed as mentioned in Materials and Methods. The elution and SDS−PAGE profiles of a reversed HPLC are shown in Fig. 7.

DNA−binding protein assays.

Assay 1: Western blots containing sl as shown in Fig. 5 were probed with digoxygenine-labeled chromosomal DNA or incubated in TBS alone (Fig. 8a). The 9 kDa protein had strong reactivity, and the 12 kDa protein showed a weak reaction. Two protein bands, corresponding to phosphorylase b and carbonic anhydrase, showed nonspecific positive reactions in the labeled and TBS control experiments.

Assay 2: The result of the band−retardation technique is shown in Fig. 8b. The mixture of the supercoiled plasmid pBR322 DNA and the 9 kDa protein purified from the strain K23−7 is shown to run slower than the control plasmid DNA. Depending on the increased ratio of protein to DNA, DNA band appears getting more tight. A specimen prepared under the same condition as shown in lane 2 in Fig. 8b was examined under EM (Fig. 8c). Note the role of the 9 kDa protein in packaging and in thickening of the DNA filament. Thus the 9 kDa protein was shown to bind DNA both of chromosomal and plasmid origins. Here, the 9 kDa protein was termed HVc.

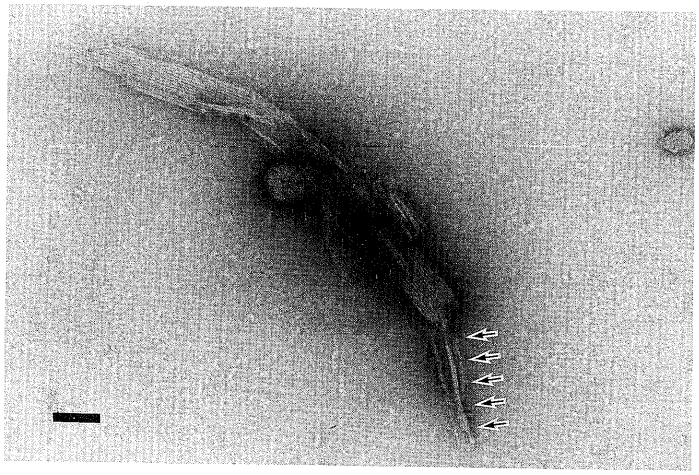

Fig. 6. An electron micrograph of the pellet taken from p2 (Fig. 5) A fine filamentous structure assembled by protofibrils with a regular width. Note that some of the filaments exhibit signs of folding (shown by arrows). Bar indicates 100 nm.

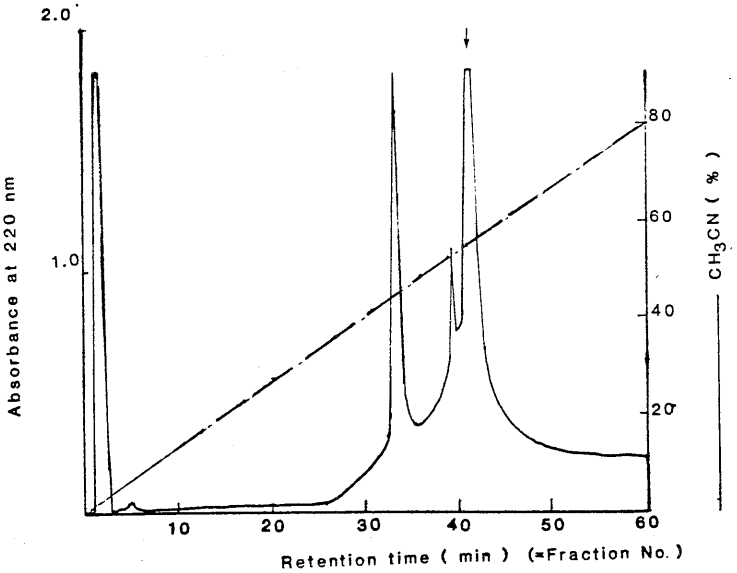

Fig. 7a. Reversed-phase HPLC profile of a DNA−binding protein from strain Chiba 3.

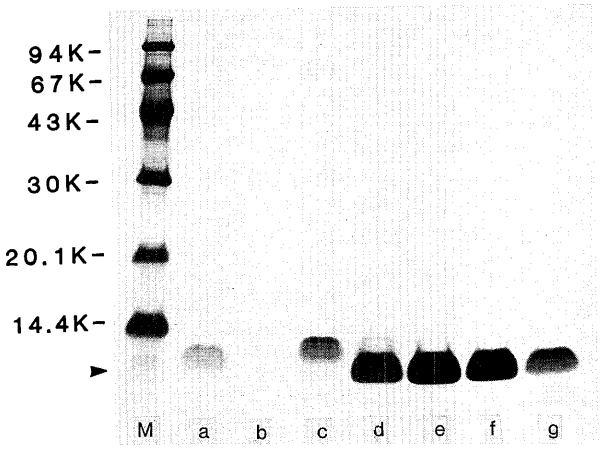

Fig. 7b. SDS−PAGE analysis of fractions eluted from C18 reversed−phase HPLC. Lanes: M; LMMPs, a to g; fractions, 34, 35, 40, 41, 42, 43, 44, respectively.

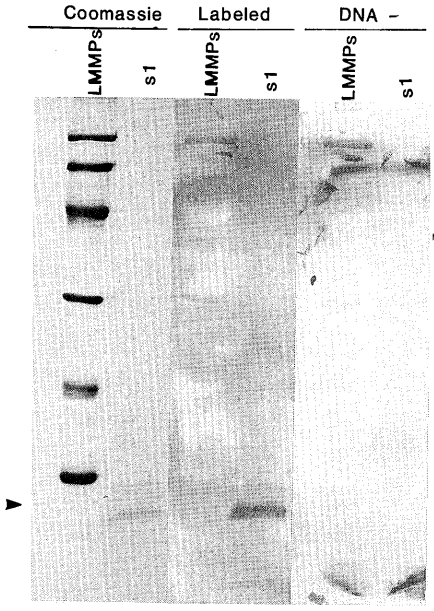

Fig. 8a. DNA−binding protein assay (1). A slab gel run with LMMPs and sl (Fig. 5) was stained with Coomassie Brilliant Blue R−250 (Coomassie) and Western blots made from similar gels were probed with digoxigenin-labeled DNA (Labeled) or incubated in TBS buffer lacking in DNA (DNA−). LMMPs: low molecular weight marker proteins. sl: used as a crude DNA−binding protein. Note that the clear reactive sl band at 9 kDa bound dsDNA probe.

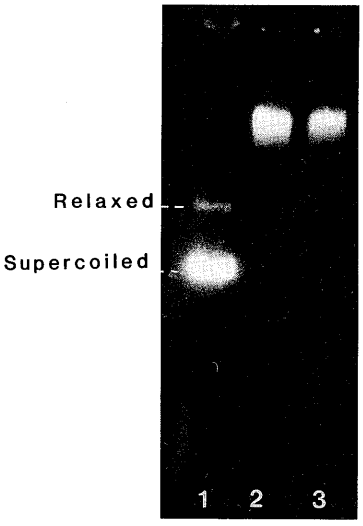

Fig. 8b. DNA−binding assay (2). Agarose gel electrophoresis of pBR322 DNA complexes formed with the 9 kDa protein purified from strain K23−7. Lanes: 1, pBR322 DNA (0.5 μg); 2 and 3, DNA at increasing ratios of protein to DNA (w/w)=2 and 4. Note that supercoiled pBR322 DNA complexed with protein seems to be retarded.

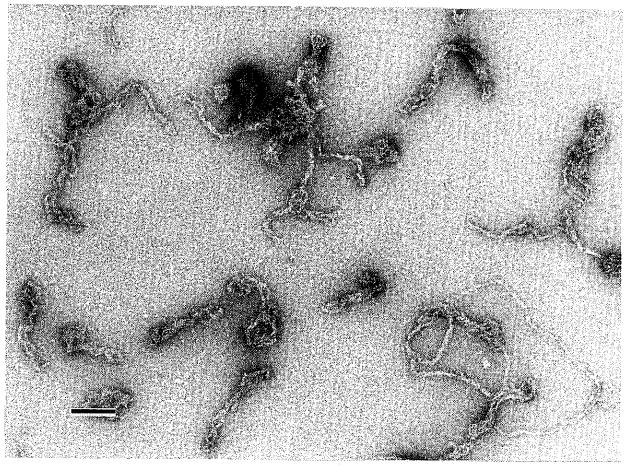

Fig. 8c. A picture of plasmid pBR322 and HVc protein complex. Note the role of HVc protein in packaging and in thickening of the plasmid DNA filament. Bar indicates 100 nm.

Ouchterlony immunodiffusion and western blot analyses of the HVc proteins. An immunodiffusion analysis is shown in Fig. 9a. Each precipitin line is shown completely fused. A western blot analysis is shown in Fig. 9b. The HVc proteins purified from the two strains were clearly detected in a western blot. The HVc protein bands were not detectable in the shear-fractions simply because of the low concentration of protein loaded.

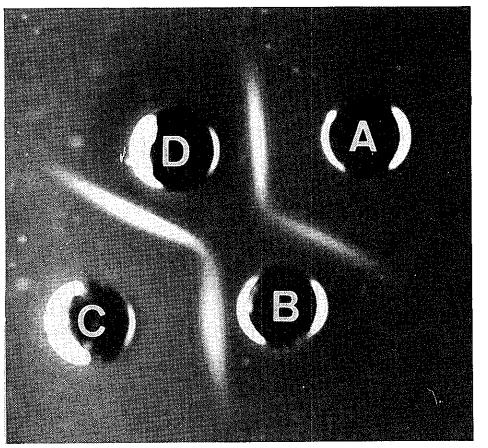

Fig. 9a. Ouchterlony immunodiffusion analysis of HVc proteins purified from *V. cholerae* O1 strains of K23−7 and Chiba 3. Wells: A and C, anti−HVc protein antisera developed against HVc protein purified from strains of K23−7 and Chiba 3, respectively; B and D, HVc protein purified from strains of K23−7 and Chiba 3, respectively.

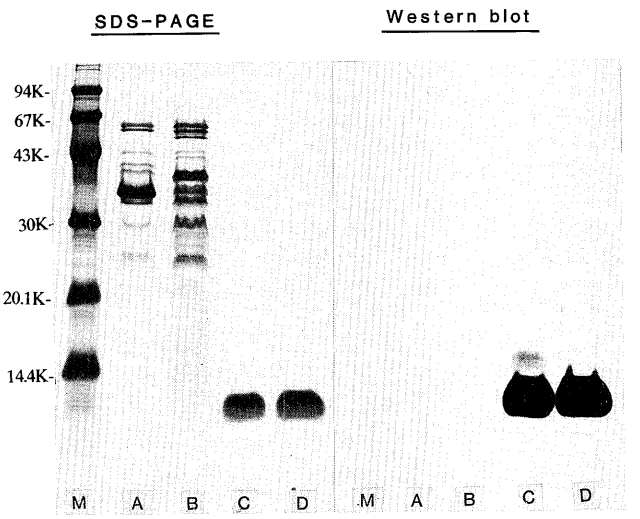

Fig. 9b. SDS−PAGE and Western blot analyses of HVc proteins derived from strains of K23−7 and Chiba 3. Lanes: M, LMMPs; A and B, shear-fractions of strains of K23−7 and Chiba 3; C and D, HVc proteins purified from strains of K23−7 and Chiba3, respectively.

Western blot analysis of the HVc proteins among vibrio strains with different pathogenicity and lysogenicity. Based on the pathogenicity and on the lysogenicity, the concentration of the HVc protein per cell was compared. Apparently as shown in lane 2 (Fig. 10), the HVc protein band of the K23−7 strain (pathogenic, lysogenic) was stained most densely, and the strain 34/D13 (non-lysogenic, lane 4), most faintly. In the western blot, the purified HVc protein band is shown to run faster than the others, possibly due to deglycosylation during the purification process.

N−terminal amino acid sequence of the HVc proteins. The N−terminal amino acid sequences of the HVc proteins purified from strains of K23−7 and Chiba 3 were identical as shown in Fig. 11. The determination of the sequence was repeated twice for each preparation. The N−terminal amino acid sequence of the HVc protein showed a high degree of homology with that of HU−2 of *E. coli,* suggesting that they originate from a common ancestral protein.

Localization of HVc protein. Immunoelectron micrographs revealed the localization of HVc proteins. HVc proteins were closely related with the bulk of DNA in broken cells and were shown to be localized at the cell-surface forming capping-like structure in relatively intact cells.

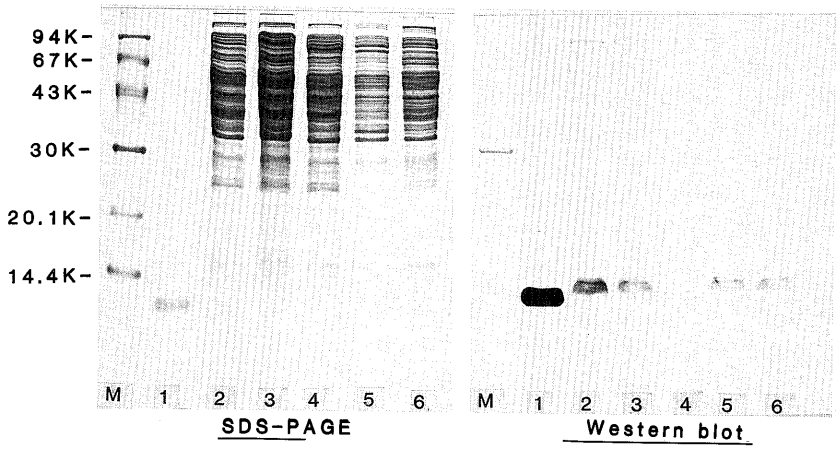

Fig. 10. SDS−PAGE and Western blot analyses of HVc proteins among *V. cholerae* O1 strains with different prophage typing. Lanes: M, LMMPs; 1, purified HVc; 2, K23−7 (Celebes original); 3, Chiba 3 (cured); 4, 34/D13; 5, U13 (Ubol); 6, U38 (Ubol). ( ): prophage typing. Note that the band for HVc protein of the phage-non-producer strain, 34/D13 was undetectable and the band for HVc protein of K23−7 strain was stained most densely.

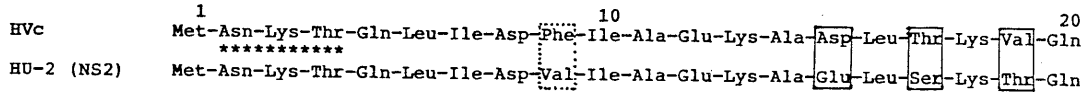

Fig. 11. Comparison of the N−terminal amino acid sequences of proteins HVc and HU−2 (NS2). Asterisks (***) indicate the glycosylation signal sequence. Solid boxes contain amino acids whose codons differ by one base change. Codons for amino acids in dotted box differ more than one base.

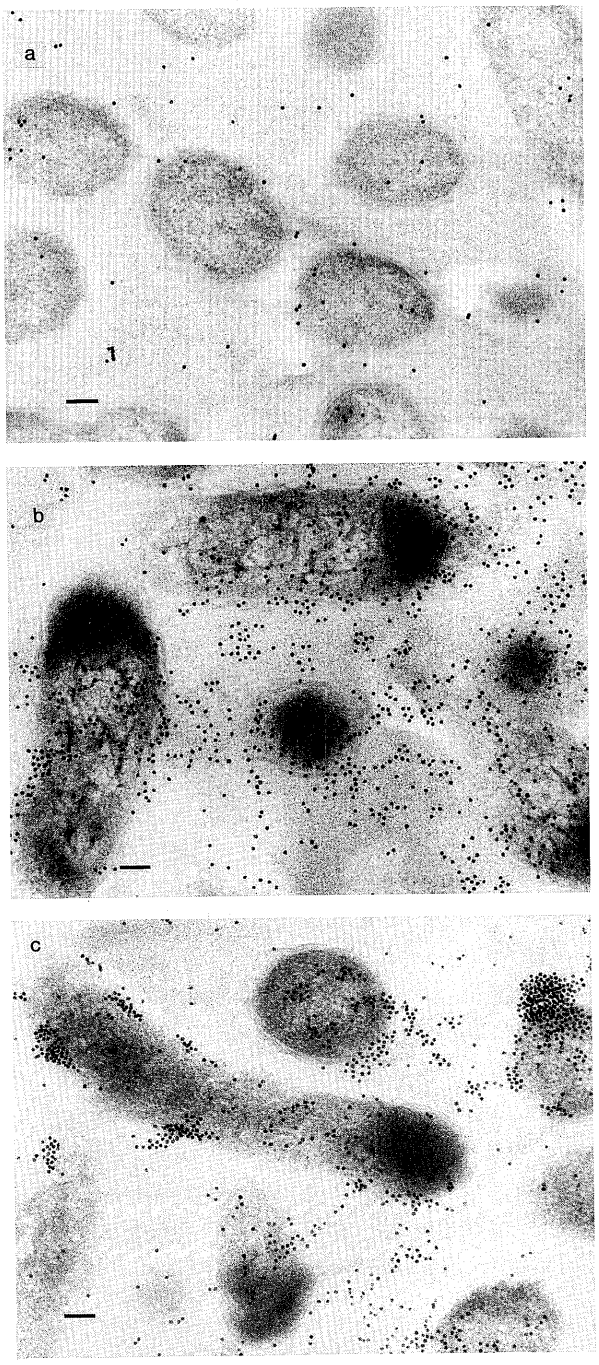

Fig. 12. Localization of HVc protein in *V. cholerae* O1, strain K23−7. Pictures: a, negative control; b, broken cells; c, relatively intact cells. Note the association of HVc protein with the bulk DNA (DNA filament in the nucleoid) in Fig. b, and the capping-like structures at the cell-surface shown in Fig. c. Bars indicate 100 nm.

Discussion

We purified a 9 kDa protein from the shear-fraction during the purification of fimbriae of *V. cholerae* O1. The N−terminal amino acid sequence of the 9 kDa protein of *V. cholerae* O1 was highly homologous to that of HU−2 of *E. coli.* Furthermore, the 9 kDa protein was confirmed to bind to DNA by two independent assay systems. We therefore designated it HVc. The role of the HVc in packaging and in thickening of the DNA filament has clearly been demonstrated. These results support that HU−2 is highly conserved throughout the entire prokaryotic kingdom, as the case of histones in eukaryotic cells. Close to the *N*−terminal of the HVc protein was seen the Asn−X−Thr sequence, a well known glycosylation site. The HVc protein appeared as a doublet band as shown in Figs. 1 and 2, probably due to the difference in glycosylation. This difference seemed to disappear after repeated treatment with 6 M guanidine−HCl as shown in Figs. 3, 4 and 5. The filamentous structures observed in the presence of ATP, GTP and Taxol as shown in Fig. 6 require further study for their functional meaning and localization. Taxol is an alkaloid which facilitates tubulin polymerization in eukaryotic cells. Therefore this observation may suggest the presence of some acidic proteins similar to tubulin in vibrio cells. Localization of the HVc protein was not well defined in this study, however close association with the bulk DNA and capping-like structure was demonstrated in EM pictures. In *E. coli,* the histonelike protein HU was not associated with the bulk DNA in the nucleoid but was located in areas of the cell where metabolically active DNA is associated with ribosomes and where single-stranded DNA, RNA polymerase, and DNA topoisomerase I were also located (Durrenberger *et al.,* 1988). A recent study by Shellman and Pettijohn (1991) clearly indicates that HU protein having normal DNA−binding and supercoiling activities associates with the nucleoid *in vivo.*

An apparent difference was seen in the number of the HVc protein molecule per cell between strains of lysogenic K23−7 and non-lysogenic 34/D13 as seen in *Neisseria gonorrhoeae* between strains of transformation-competent and DNA uptake-deficient mutant (Doward and Garon, 1989). Further studies are required to realize the correlation between the number of HVc protein molecule per cell and pathogenicity, and/or lysogenicity.

It should be noted that CS resin is valuable for the purification of HU−like proteins because it is easy to use and gives reproducible results.

Acknowledgments

We thank Katsutoshi Aihara, The Institute of Chemo-Sero Therapeutic Research, Kumamoto, Japan, for introducing us the procedure of Cellulofine Sulfate affinity chromatography.

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