VIROLOGY **206, 520-526 (1995)**

Coat Protein of the Ectocarpus siliculosus Virus

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Received June 24, 1994, accepted October 31, 1994

Ectocarpus siliculosus **virus, EsV, multiplies in sporangia and gametangia of the marine brown alga** Ectocarpus siliculosus. **We describe an improved method for the isolation of morphologically intact and infectious virus from diseased plants. We show that treatment of virus particles with high concentrations of CsC1 results in a substantial loss of structural proteins. One of the proteins which resists CsC1 treatment is glycoprotein-1, the largest of the three viral glycoproteins. We have isolated an EsV genomic fragment with an open reading frame encoding glycoprotein-1 . The predicted amino acid sequence is rich in hydrophilic amino acids, but contains hydrophobic regions close to the amino and carboxy termini. A discrepancy between the molecular weight predicted from the coding region and the molecular weight determined by gel electrophoresis suggests that proteolytic processing is required for the maturation of the protein.** 10 1995 Academic Press, Inc.

INTRODUCTION

Ectocarpus siliculosus is a cosmopolitan benthic filamentous marine brown alga. It inhabits coasts in temperate climate zones of both hemispheres. Representative plants from many localities carry symptoms of an infection caused by a latent virus, the Ectocarpus siliculosus Virus, EsV (Müller *et al.,* 1990; Müller and Stache, 1992).

Induction and multiplication of EsV occurs in the reproductive organs of the host. EsV genomes replicate and assembie as closely packed viral particies in prospective gametangia and sporangia and are eventually discharged into the surrounding seawater (Müller, 1991a). Free virus particles are infective for a few days (Müller and Frenzer, 1993) and can invade swimming gametes or spores of healthy plants, but cannot infect somatic *Ectocarpus* cells which are protected by their solid cell wall (Müller *et aL,* 1990). Infected gametes or spores develop into infected plants, implying that all cells of the progeny plant carry the virus genome, but permit virus induction and multiplication only in the prospective reproductive cells. In genetic crosses between healthy and infected gametophytes, the EsV genome is transmitted like a Mendelian trait as meioses produce equal numbers of healthy and infected spores (Müller, 1991b).

Thus, EsV in its natural environment remains infectious for short time periods, easily invades the host via its gametes or spores, and can be eliminated by meiosis. These are features of a noniethal, weil balanced hostpathogen system with interesting evolutionary and ecological implications.

Previously, we isolated EsV particles from infected plants aiid found that the viral genome is double stranded DNA of 320 kb which is isolated in linear and circular forms (Lanka *et al.,* 1993). Our isolation procedure involved a precipitation of virus particles from disrupted infected plants by polyethylene glycol followed by equilibrium centrifugation in CsC1 gradients. EsV particles, purified by this procedure, were not infectious (in contrast to virus particles spontaneously released from infected cells into seawater; Müller *et aL,* 1990). They also differ in their electron microscopic appearance from those seen in infected plant cells (Lanka *et al.,* 1993).

In the present communication, we describe an improved isolation procedure replacing the CsC1 step with Percoll gradient centrifugation. This procedure yields morphologically intact EsV particies that readily infect unicellular zoospores which then develop into mature plants showing pathological symptoms. As one explanation for the lack of infectivity of CsCI-purified EsV we found that CsC1 induces the dissociation of several viral coat proteins. We have determined properties of one coat protein, glycoprotein 1, as a first step for investigating the complex architecture of EsV.

MATERIALS AND METHODS

Cells

Stocks of clonal sporophyte cultures of E. siliculosus were maintained bacteria-free on 1% agar in culture medium (autoclaved seawater with ES enrichment according to Starr and Zeikus, 1993). Mass cultures were initiated by inoculation of agar-grown fragments into liquid medium in Petri dishes which were later expanded to 0.5-liter cultures. Conditions for cultivation were as previously described (Lanka et al., 1993).

The virus-free strain is a female parthenosporophyte derived from a native New Zealand gametophyte (code number NZ 4a3 f).

For virus production we used the previously described E. siliculosus clone NZ-Vic-Z14 which develops symptoms of viral infection when transferred from agar to liquid medium (Lanka *et* al., 1993).

Preparation of virus particles

Virus particles were isolated from infected plants and collected by precipitation in polyethylene glycol (PEG) as previously described (Lanka *et* al., 1993). The PEG pellet was resuspended in 0.5 ml seawaler/0.05 M Tris-HCl (pH 7.8) and mixed with a Percoll solution (Sigma) to give a final concentration of 80% Percoll (in seawater/0.05 M Tris-HCl, pH 7.8). The solution was centrifuged to equilibrium for 1 hr at 65,000 g and 4' in a fixed-angle rotor. The turbid virus band was collected by puncturing the tube with a hypodermic needle.

Electron microscopy

Isolated virus particies were mounted on carboncoated grids which had been exposed to glow discharge of tripropylamine vapor (Dubochet and Groom, 1982). The grids were then treated with uranyl acetate (Haschemeyer, 1970). Micrographs were taken in a Zeiss EM 900 electron microscope.

Molecular cloning

We have restricted EsV DNA with the endonucleases EcoR1 and Sall. The resulting fragments were cloned into plasmid vector pUC 9. Several of the cloned virus DNA fragments were sequenced by the chain termination method of Sanger *et* al. (1977). A 0.93-kb fragment contained an open reading frame, and computer analysis reveaied some limited degree of similarity to known viral coat proteins (see below). The 0.93-kb fragment was used as a probe to isolate a 2.3-kb BamH1 fragment from an EsV genomic library (see below). Part of this DNA fragment was subcloned in plasmid pRSET (Invitrogen/ ITC Biotechnology) and induced for expression after transfection into bacteria as suggested by the supplier. The overexpressed protein was purified from bacterial extracts and used to immunize rabbits (Harlow and Lane, 1988).

For preparation of a genomic library, the EsV genome was partially digested with the restriction endonuclease Sau3A, and DNA fragments of 9-23 kb were cloned in the BamH 1 sites of vector X-G EM 12 (Promega) accord i ng to standard procedures (Sambrook *et* al., 1989).

Protein analysis

Virus particles, obtained after Percoll centrifugation, were pelleted at 80,000 g and resuspended in loading buffer. Proteins were electrophoresed on polyacrylamide gels in the presence of sodium dodecyl sulfate (SDS) (Laemmli, 1974). The separated polypeptides were visualized by silver staining (Wray *et* al., 1981) or transferred to a nylon membrane for the staining of glycoproteins (Gershoni *et* al., 1985) or for immunostaining (Western blotting; Towbin *et* al., 1979). Immunostaining of meinbrane-bound proteins was performed with rabbit antibodies specific for the bacterially expressed EsV coat p rotein.

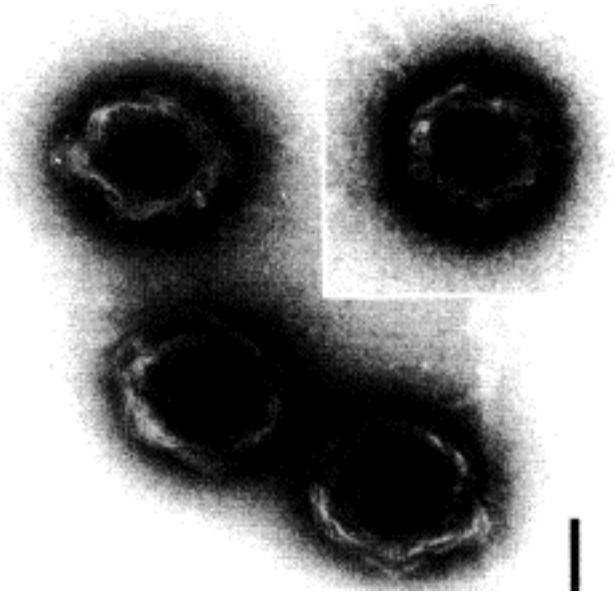

FiG. 1. Morphology of isolated EsV. The multilayered structure corresponds to ihat previously described for densely packed intracellular virus parlicles (see Müller *et al., 1990;* Lanka *et al., 1993).* Bar, 0.1 um.

RESULTS

Isolation of infective virus particles

Previously, we purified PEG-precipitated virus particles by CsC1 gradient centrifugation (Lanka *et* al., 1993). These virus preparations were not infectious in contrast to native virus particles reieased from infected plants (Müller *et* al., 1990). We concluded that viral particles were damaged by the high ionic

strength of CsC1 solutions and consequently tried Percoll gradients as an alternative final purification step. Indeed, electron microscopic examination revealed that EsV particles recovered after the Percoll step were morphologically similar (Fig. 1) to the viral structures seen in EM sections of plant cells (Müller *et* al., 1990; Lanka *et* al., 1993).

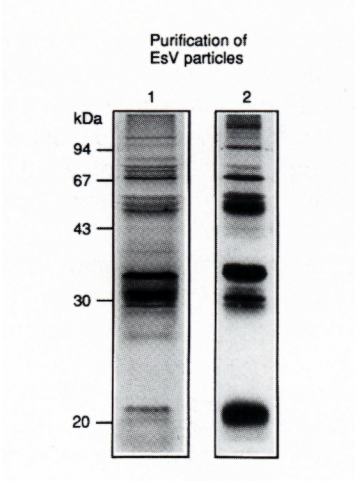

FiG. 2. Structural proteins of EsV purified by centrifugation in a CsC1 gradient (1) and in a Percoll gradient (2). About 5 Mg protein each was electrophoresed on a 12% polyacrylamide gel and visualized by silver staining.

We have performed infection experiments to test whether Percoll-purified EsV retains its biological activity. As a recipient e used symptom-free parthenosporophytes with mature plurilocular sporangia. These plants ere induced to release their spores by transfer to fresh culture medium and a temperature increase from 4 to **180 (Müller, 1991a).** Aliquots of 200-300 free-swimming spores ere dispensed into 0.5 ml medium in 5-cm plastic dishes. Aliquots of the EsV preparation ere then added and gently stirred. After 1 hr the zoids had settled at the bottom of the plastic dishes. Fresh medium (10 ml) was then added to each dish and incubation continued at 18' for 2 weeks. Depending on the amounts of virus added, beteen 10 and 35% of the developing plants shoed the symptoms characteristic for virus infection (Müller *et al.,* 1990). Mock infection experiments with parallel cultures never produced pathological symptoms.

These experiments fulfill Koch's postulates: the infectious agent as isolated from a diseased organism and used to infect a healthy recipient hich thereafter developed the same symptoms originally observed in the field sample.

Infection experiments ith CsCI-treated virus preparations were alays negative, and one reason forthis could be a loss of structural proteins during CsC1 gradient centrifugation. Gel electrophoretic analyses revealed qualitative differences betv\/een the to virus preparations. As shown in Fig. 2, polypeptides of about 35 and 21 kDa molecular eight are the most prominent protein constituents of Percoll-purified EsV, but they are far less abundant in CsCI-treated virus preparations. In addition, CsC1purified preparations almost entirely lack several polypeptides of molecular eight larger than 70 kDa (Fig. 2).

We initiated an analysis of the EsV coat and investigated some properties of a major viral structural protein.

The EsV glycoprotein-1

We have cloned and sequenced a 2.3-kb piece of EsV DNA. This DNA seqment hybridizes ith the 65-kb Ascil

Sfil fragment of the EsV genome (Lanka *et al.,* **1993) and** is included in a 13.7-kb fragment of an EsV genomic library construcied in the lambda-derived vector XGEM12. These findings allo a tentative assignment of the 2.3-kb fragment to the EsV genome as shown in Fig. 3.

The 2.3-kb fragment does not hybridize to DNA from uninfected E. siliculosus cells or to DNA from a virus infectious for the marine bron alga *Feldmannia* (Henry and Meints, 1992; Friess-Klebi *et al.,* 1994, data not sho\/vn).

The fragment contains an open reading frame of 1983 bases. The region (50 bp) upstream of the initiation codon is rich in AT base pairs (not shon) ith an AT content of 63% compared to 50% in the open reading frame. Interestingly, AT- rich upstream regions \/vere also found in genes from *Chlorella* viruses and may have a function in gene expression (Schuster *et al.,* 1990; Graves and Meints, 1992).

In Fig. 4A, we sho the amino acid sequence deduced from the open reading frame of the 2.3-kb EsV DNA fragment. The amino acid sequence corresponds to a polypeptide ith a calculated molecular weight of about 72 kDa. The sequence includes four amino acid motifs frequently found at sites modif ied by N-glycosylation (boxed in Fig. 4A) (Hart *et al.,* 1979). The predicted polypeptide is rich in hydrophilic amino acids with a calculated p/ of 4.2, but there are two hydrophobic domains close to the

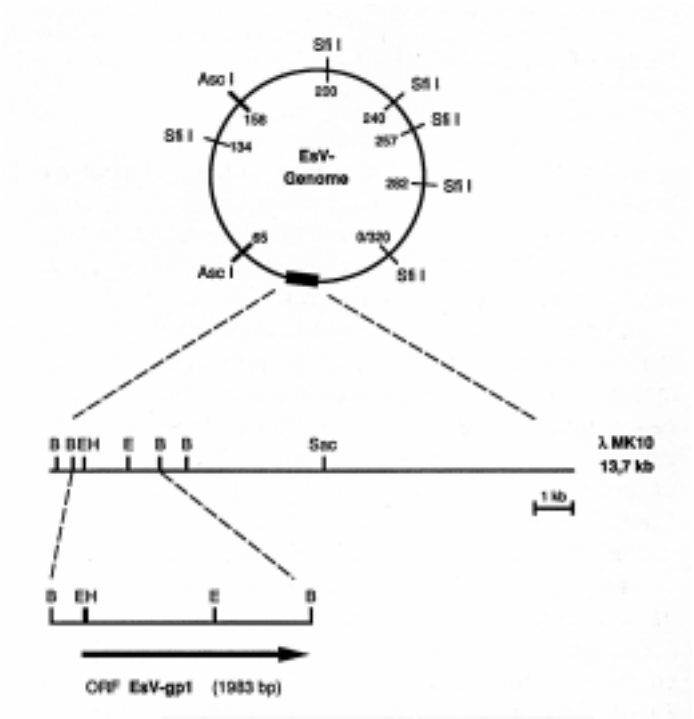

FIG. 3. Mapping of ihe EsV-gpl gene. (Top) Restriction map of the EsVgenome (Lanka *etal., 1993).* (Bottom) InsertofphagecloneXMK10 (restriction sites.- B, *BamHI;* E, EcoRI; *H, HindIII) containing* an open reading frame (ORF) of *1983* bp encoding ihe EsV glycoprotein-1 (gpl). Arrow, direction of transcription.

FiG. 4. Predicted amino acid sequence of EsV glycoprotein-i. (A) Amino acid sequence. Underlined regions are rich in hydrophobic (straight line) or hydrophilic (broken line) amino acids. Potential Nlinked glycosylation sites are boxed. (B) Hydrophilicity plot. (C) Regions with limited similarities t o knov\/n viral and cellular proteins (see text). The EsV nucleotide sequence has been deposited with the EMBL data library under Accession No. X76296.

aminoterminal end and the carboxylerminal end of the polypeptide (Fig. 4B). Application of the ruies of Chou and Fassman (1978) predicts a high content of ß strands as a major secondary structure element for the entire amino acid sequence (not shown).

Computer-assisted sequence comparisons reveaied that the extreme hydrophobic amino terminal section (homology region 1 in Fig. 4C) of the predicted sequence has a limited similarity (29% identities in 24 amino acids) with ihe flavivirus encoded glycoprotein NS1 that is found on the surface of flavivirus-infected cells (Hahn *et al.,* 1988). The sequence of hydrophobic amino acids, included in homology region 2 of Fig. 4C, is similar (39% identity in 23 amino acids) to a hydrophobic aminoterminal part of an envelope protein of hepatitis C virus (Takamizawa *et al.,* 1991), and the more centrally located stretch of amino acids 140 to 230 (homology region 3, Fig. 4C) shares 20 of 90 amino acids with an external part of a mammalian membrane-associated surface glycoprotein (Brümmendorf *et al.,* **1989).** Finally, computer analysis indicates that the extreme carboxyterminal hydrophobic reqion (Fiq. 4B) of the predicted amino acid

sequence is similar to the transmembrane domains of a number of membrane-associated proteins as diverse as apoprotein p680 of photosystem 11 from different plants (33% identity in an 18-amino-acid sequence) (OffermannSteinhard and Herrmann, 1990) and neural cell adhesion glycoproteins (36% identity in 33 amino acids) (Miura *et al.,* 1991).

Even though the similarities to other viral and cellular membrane proteins are limited, they could indicate that the open reading frame of Fig. 4A may encode an EsV coat protein. We therefore expressed the major part of the open reading frame (the *HindIII-BamH1 subfragment* of Fig. 3) in bacteria and used the baclerially expressed and purified polypeptide as an antigen to raise antibodies in rabbits.

The specificity of the anlibodies was tested with extracts from bacteria expressing the EsV sequence. The EsV antibodies but not preimmune antibodies specifically reacted in Western blots with a polypeptide expressed in bacteria induced to synthesize the EsV open reading frame (Fig. 5). The apparent molecular

weight of the immunoreactive polypeptide is close to 100 kDa, significantly higher than the molecular weight as calculated from the predicted amino acid sequence. This discrepancy may be due to high local concentrations of negatively charged amino acids (see Fig. 4B) which could prevent the binding of sufficient amounts of sodium dodecyl sulfate (Matagne *et* al., 1991).

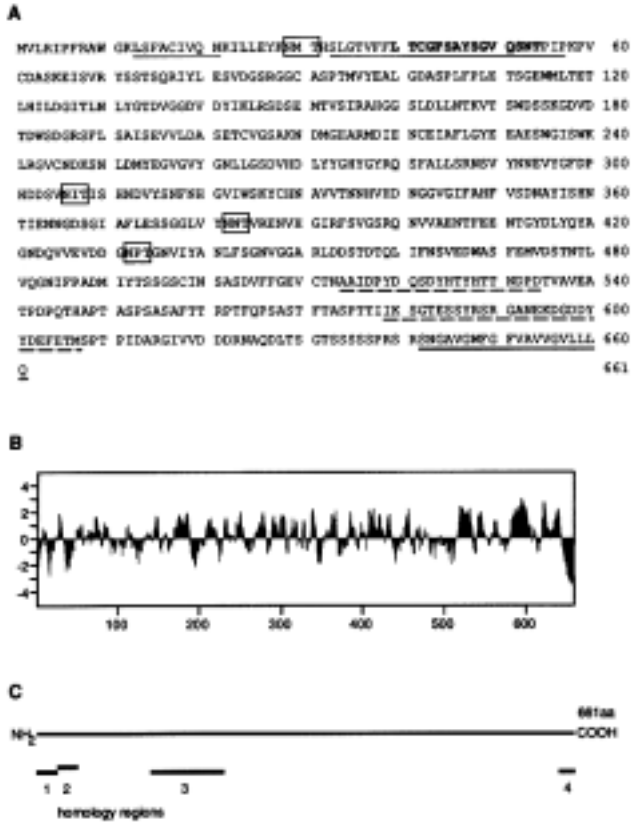

FiG. 5. Bacterial expression of the EsV gpl-sequence. (Left) The open reading frame between the ***HindIII and*** BamH1 restriction sites as shown al the bottom of Fig. 3 was linked to the multiple cloning site of expression vector pRSET. We show a silver-stained polyacrylamide gel (8%) with equal amounts of proteins from uninduced and induced bacteria. (Right) The bacterially expressed EsV-protein was used as an antigen to raise anlibodies in rabbits. Proteins from induced bacteria were electrophoretically separated on polyacrylamide gels (10%), blotted onto nylon membranes, and probed with EsV-protein antibodies or with preimmune control antibodies as indicated (Towbin et al., 1979).

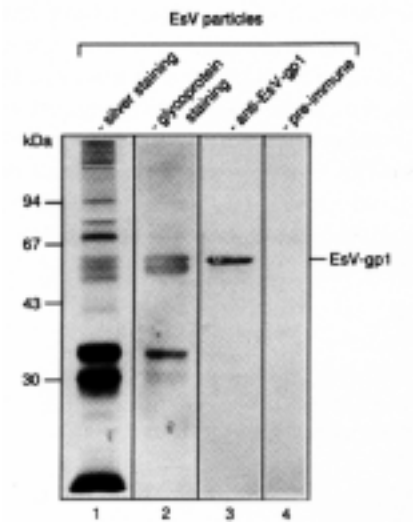

FiG. 6. Identification of glycoprotein-1 as a viral structural protein. Percoll-purified EsV was disrupted in SDS and investigated by denaturing polyacrylamide gel (10%) electrophoresis (Laemmli, 1974). Lane 1, silver staining; lane 2, blotting on nylon membrane and staining according to Gershoni et al. (1985) for ihe identification of glycoproteins; lane 3, blotling and probing with EsV protein-specific antibodies; lane 4, probing with preimmune control antibodies (see Fig. 5).

We have directly determined the presence of the protein in EsV parlicles as prepared by ihe Percoll procedure. Viral particles were disrupted in the presence of ß-mercaptoethanoi and SDS and investigated by denaturing gel electrophoresis (Laemmli, 1974). We added identical aliquots of the SDS-treated virus preparation to several adjacent lanes of the polyacrylamide gel and stained one lane with silver salts (lane 1, Fig. 6). The proteins in other lanes were transferred to nylon meinbranes for the identification of glycoproteins and for immunoreactions with specific and preimmune control antibodies. Using the enzyme-hydrazide method of Gershoni et al. (1985) we could identify three glycoproteins with apparent

molecular weights of about 60, 56, and 35 kDa (lane 2, Fig. 6). The specific antibodies strongly reacted with the largest of the three EsV glycoproteins (lane 3, Fig. 6). We refer to this protein as EsV glycoprotein-1 or EsV-gpl.

The reiationship between glycoprotein-1 and the bacterially expressed protein was supported by biochemical means using endoproteinase Lys-C. Proteolytic fragments of identical electrophoretic properties were obiained after Lys-C treatment of glycoprotein 1 (eluted from a polyacrylamide gel; see Fig. 6) and of the bacterially expressed polypeptide (Figs. 5, 7).

We conclude that the open reading frame of 1983 bases (Fig. 4) corresponds to the EsV gene-encoding viral glycoprotein-1.

There is, however, an important discrepancy between the apparent molecular weight of 60 kDa, electrophoretically determined for the mature glycoprotein-1, and the molecular weiqht of 72 kDa, predicted from the open

reading frame. Presently, we cannot explain this difference, but processing of a precursor translation product may be a realistic possibility (Kräussl-ich and Wimmer, **1988).** In fact, we could identify in the aminoterminal part of the predicted gpl sequence a signal sequence cleavage site, characteristic for proteins which are processed during the passage of membranes (Fig. 4) (Heijne, 1986). However, further experiments must show whether this site is used forthe processing of glycoprotein 1, or, more generally, how the mature viral structural protein is related to its larger precursor protein.

We have incubaled Percoll-purified EsV particies with the gpl -specific antibodies to determine possible eff ects on viral infectivity. The results obtained were inconclusive: we observed an antibody-specific reduction of infected plants with some viral preparations, but no effects of the antibody on the infectivity of other preparations. A possible explanation may be that the presence of viral glycoprotein-1 (or its antigenic part) on the surface of viral particles varies with different preparations, depending, for example, on the status of virus assembly or maturation at the time when plants are disrupted for the isolation of virus. This possibility is supported by a comparison of the structural proteins in the virus preparation used for ihe electrophoretic analysis in Fig. 2 (lane 2) with those in the virus preparation in Fig. 6 (lane 1): the two independent virus preparations contain identical protein components, but in different relative amounts.

DISCUSSION

We have described an improved method for isolating EsV from infected and diseased algae. Using this method, we avoid an exposure of PEG-precipitated virus to the high ionic strength of CsC1 solutions and use

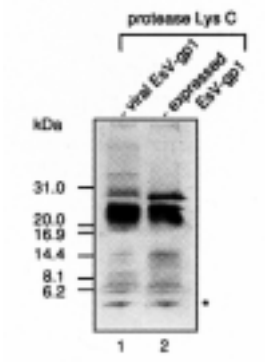

FIG. 7. Proteolysis. Glycoprotein 1 was identified on a Laemmli gel as shown in Fig. 6 (lane 1). The corresponding gel slice was excised and added t o a slot of a Tricine-SDS gel (Schägger and von Jagow, 1987). A parallel lane received 1.5 Mg of the bacterially expressed protein. Proteins in both lanes were then treated with 1 Mg protease Lys C (Boehringer Mannheim) for 30 min. Electrophoresis was performed as described (Schägger and von Jagow, 1987). Peptides were visualized by silver staining. Asterisk, polypeptide from the proteinase preparation used.

instead Percoll-centrifugation as the final purification step. The improved method yields viral particles with higher protein/DNA ratios than those of CsCI-purified viruses. It appears therefore that high CsC1 concentrations disrupt the viral protein shell. We found that treatment with CsC1 interferes with the biological activity of EsV.

Percoll-purified viruses are composed of at least 16 electrophoretically detectable structural proteins. Some of these proteins are rather intensely stained by silver salts and are therefore present in many copies per particle (Figs. 2, 6). These abundant proteins are most likely the major building blocks forming the characteristic twolayered shell of densely packed virus particles in the infected cell (Müller *et al., 1990)* and of isolated infective viruses. But we have no information yet concerning the distribution of these proteins between the thin outer layer and the broader, more diffuse inner layer of an EsV particle (Fig. 1).

It could be assumed that the three viral glycoproteins, identified by the specific staining procedure of Gershoni *et al.* (1985), may be components of the outer shell because glycoproteins are frequently found as constituents of viral surfaces. However, viral glycoprotein-1 as well as glycoprotein-2, the second largest EsV glycoprotein, were also detected in CsCl-purified viral structures (Fig. 2; Lanka *et al.,* 1993) and may therefore be located in an inner protected part of the viral particle. In contrast, the third glycoprotein (apparent molecular Weight: 35 kDa), which is much more abundant than glycoproteins-1 and -2 in native virus preparations (Fig. 6), was not detected in CsCI-purified EsV (Lanka *et al.,* 1993) and could therefore be located al ihe viral surface.

As a first step in analyzing the genetics of EsV, we identified an open reading frame that could encode glycoprotein-1, the largest of the three EsV glycoproteins. Our identification relies on two pieces of evidence: first, antibodies against the bacterially expressed polypeptide strongly and specifically react with glycoprotein-l; second, in *vitro* proteolysis produces identical peptides after treating the bacterially expressed polypeptide and glycoprotein-1 with endoproteinase Lys-C. In addition, the predicted amino acid sequence contains several motifs known to be sites for N-glycosylation. The sequence has also several features commonly found in membrane-associated cellular and viral glycoproteins. This includes a hydrophobic carboxyterminal domain characteristic for proteins integrated in a lipid bilayer. Presently, however, we do not know whether the protein is anchored to a lipid membrane.

Determination of the nucleotide sequence in and around the gpl-gene gives a first glimpse at an EsV gene structure. The open reading frame is not interrupted by introns, and the upstream region of the gpl-gene is unusually rich in AT base pairs quite similar to the correspondinq reqion of a *Chlorella* virus coat protein qene

(Graves and Meints, 1992) but the implications for gene expression and regulation have yet to be worked out.

From a more practical point, the known sequence of a piece of the EsV genome offers the opportunity to use PCR assays, which will be of great value in investigating the distribution of EsV in algal populations from natural habitats and in obtaining important new information concerning the ecological consequences of EsV induction and infection.

ACKNOWLEDGMENTS

We thank Uwe Ramsperger for his heip with electron microscopy and Martin Bräutigam for discussions. This work was supported by Deutsche Forschunsgemeinschaft.

REFERENCES

BRÜMMENDORF, T., WOLFF, J. M., FRANK, R., and RATHJEN, F. G. (1989). Neural cell recognition molecule Fl 1: Homology with fibronectin type lil and immunoglobuline type C domains. *Neuron* 2, 1351 -1361.

CHOU, P. Y., and FASMAN, G. D. (1978). Empirical predictions of protein conformation. *Annu. Rev. Biochem.* 47, 251 -276.

DuBOCHET, J., and GROOM, M. (1982). The mounting of macromolecules for electron microscopy with particular reference to surface phenomena and the treatment of support films by glow discharge. *Adv. Opt Elect* Microc. 8, 107-135.

FRIEss-KLEBL, A. K., KNIPPERS, R., and MÜLLER, D. G. (1994). Isolation and characterization of a DNA virus infecting *Feldmannia simplex* (Phaeophyceae). *J. Phycol. 30,* 653-658.

GERSHONI, J. M., BAYER, E. A. and WILCHEK, M. (1985). Blot analyses of glycoconjugates: Enzyme-hydrazide, a novel reagent for the detection of aldehydes. *Anal. Biochem. 146, 59-63.*

GRAVES, M. V., and MEINTS, R. H. *(1992).* Characterization of the major capsid protein and cloning of its gene from algal virus PBCV-1. Virol*ogy 188, 198-207.*

HAHN, Y. S., GALLER, R., HUNKAPILLAR, T., DALRYMPE, J. M., STRAUSS, J. H., and STRAUss, E. G. *(1988).* Nucleotide sequence of Dengue *2* RNA and comparison of the encoded proteins with those of other flaviviruses. Virology *162, 167-180.*

HARLOW, E., and LANE, D. *(1988).* "Antibodies. A Laboratory Handbook." Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

HART, G. W., BREW, K., GRANT, G. A., BRADSHAW, R. A., and LENNARZ, W. J. *(1979).* Primary structural requirements for the enzymatic formation of ihe N-glycosidic bond in glycoproteins: Studies with natural and synthetic peptides. J. *Biol. Chem. 254, 9747-9753.*

HASCHEMEYER, R. H. *(1970).* Electron microscopy of enzymes. *Adv. Enzymol. 33, 71 -118.*

HEJNE, V. G. *(1986).* A new method for predicting signal sequence cleavage sites. *Nucleic Acids Res. 14, 4683-4690.*

HENRY, E. C., and MEINTS, R. H. *(1992).* A persistent virus infection in *Feldmannia* (Phaeophyceae). J. *Phycol. 28, 517-528.*

KRÄUSSLICH, H. G., and WIMMER, E. *(1988).* Viral proteinases. *Annu. Rev. Biochem. 57, 701 - 754.*

L-AEMMLI, U. K. *(1974).* Cleavage of structural proteins during assembly of the head of bacteriophage *T4. Nature 227, 680-685.*

LANKA, S. T. J., KLEIN, M., RAMSPERGER, U., MÜLLER, D. G., and KNIPPERS, R. *(1993).* Genome structure of a virus infecting ihe marine brown alga *Ectocarpus siliculosus. Virology 193, 802-811.*

MATAGNE, A., JORis, B., and FRI~RE, J. M. *(1991).* Anomalous behaviour of a protein during SDS/PAGE correcied by chemical modification of carboxylic groups. *Biochem.* J. *280, 553-556.*

MIURA, M., KoBAYASHI, M., Asou, H., and UYEMURA, K. *(1991).* Molecular cloning of cDNA encoding the rat neural cell adhesion molecule LI. *FEBS Lett. 289, 91 -95.*
526

MÜLLER, D. G. (1991 a). Marine virioplankton produced by infected *Ectocarpus siliculosus* (Phaeaphyceae). *Marine Ecol. Progr.* Series 76, 101-102.

MÜLLER, D. G. (1991 b). Mendelian segregation of a virus genome during host meiosis in the marine broVvn alga *Ectocarpus siliculosus. J. Plant Physiol.* 137, 739-743.

MÜLLER, D. G., KAWAI, H., STACHE, 13., and L-ANKA, S. (1990). A virus infection in ihe marine brown alga *Ectocarpus siliculosus* (Phaeophyceae). *Botanica Acta* 103, 72-82.

MÜLLER, D. G., and STACHE, B. (1992). Worldwide ocurrence of virusinfections in filamentous marine brown algae. *Helgoländer Meeresuntersuchungen* 46, 1 -8.

MÜLLER, D. G., and FRENZER, K. (1993). Virus infections in three marine brown algae: *Feldmannia irregularis, F simplex,* and *Ectocarpus siliculosus. Hydrobiologia* **260/261,** 37-44.

OFFERMANN-STEINHARD, K., and HERRMANN, R. G. (1990). Nucleolide sequences of psbB and psbH, ihe plastid encoded genes for CP47 and the lOkDa phosphoprotein of photosystem 11 in *Oenothera hookeri* and *argillicola. Nucleic Acids Res.* 18, 6452.

SAMBROOK, J., FRITSCH, E. F., and MANIATIs, T. (1989). Wolecular Cloning: A Laboratory Handbook." Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

SANGER, F., NICKLEN, S., and COULSEN, A. R. (1977). DNA sequencing with chain terminating inhibilors. *Proc. Natl. Acad. Sci. USA* 74,54635467.

SCHAGGER, H., and VON JAGow, G. (1987). Tricine-sodium dodecylsulfatepolyacrylamide gel electrophoresis for the separation of proteins in ihe range from 1 to 100 kDa. *Anal. Biochem.* 166, 368-379.

SCHUSTER, A. M., GRAVES, M., KORTH, K., ZIEGELBEIN, M., BRUMBAUGH, J., GRONE, D., and MEINTS, R. H. (1990). Transcription and sequence studies of a 4.3 kbp fragment from a dsDNA eukaryolic algal virus. *Virology* 176, 515-523.

STARR, R. C., and ZEIKUS, J. A. (1993). UTEX-The culture collection of algae al the University of Texas al Austin. *J. Phycol.* 29 (Suppl.), 1 - 106.

TAKAMIZAWA, A., MORI, C., FUKE, 1., MANABE, S., MURAKAMI, S., FuilTA, J., 0NisHi, E., ANDOH, T., YOSHIDA, 1., and OKAYAMA, H. (1991). Structure and organization of the hepatitis C virus genome isolated from human carriers. *J. Virol.* 65, 1105-1113.

TowBIN, H. T., STAEHELIN, T., and GORDON, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheels: Procedure and some applications. *Proc. Natl. Acad. Sci. USA* 76, 4350-4354.

WRAY, VV., BoULIKAs, T., WRAY, V., and HANCOCK, R. (1981). Silver staining of peoteins in polyacrylamide gels. *Anal. Biochem.* 118, 197-203.