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Nucleocapsid protein VP15 is the basic DNA binding protein of white spot syndrome virus of shrimp

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Summary. *White spot syndrome virus* (WSSV) is type species of the genus *Whispovirus* of the new family *Nimaviridae*. Despite the elucidation of its genomic sequence, very little is known about the virus as only 6% of its ORFs show homology to known genes. One of the structural virion proteins, VP15, is part of the nucleocapsid of the virus and shows homology to some putative baculovirus DNA binding proteins. These DNA-binding or histone-like proteins are thought to be involved in the condensation and packaging of the genome in the nucleocapsid. Using bacterially expressed VP15 fusion proteins in ELISA and Far-Western experiments showed that VP15 interacts with itself, forming homomultimers, but not with the other major structural proteins of the WSSV virion. Antibodies against phosphorylated proteins revealed that VP15 originating from different sources was not phosphorylated. WSSV VP15 binds non-specifically to double-stranded DNA, but has a clear preference to supercoiled DNA suggesting that VP15 is involved in the packaging of the WSSV genome in the nucleocapsid. This research shed further light on the composition of WSSV virions and the function of one of its nucleocapsid proteins.

Introduction

Since its discovery in south-east Asia in the beginning of the 1990s, white spot syndrome virus (WSSV) has developed into a devastating epidemic in shrimp. WSSV belongs to the virus family *Nimaviridae,* genus *Whispovirus* [13] and has a circular dsDNA genome of about 300 kb, coding for approximately 184 open reading frames (ORFs) [22, 28]. As there is little homology between WSSV ORFs and other known sequences in public databases combined with its unique virion structure [5], much research has focused on the structural proteins of WSSV.

The WSSV virion consists of five major- and at least 14 minor proteins [11, 19, 20, 24, 30]. Of the major structural proteins, two are located in the virus envelope

(VP19 and VP28) and three are part of the nucleocapsid (VP15, VP24 and VP26). An *in vivo* neutralization assay and vaccination experiments have shown that VP28 is important in the systemic infection of WSSV and protective immunity response of the shrimp [23, 26, 27]. For VP19, only vaccination via injection revealed a possible role for this protein in provoking a protective immune response in shrimp [27]. Several characteristics of VP15 suggest that it may be the DNA-binding or histone-like protein of WSSV. VP15 is a major structural protein located in the nucleocapsid with a theoretical molecular weight of 6.7 kDa and has a basic pI of 13.2. The amino acid sequence shares some motifs with baculovirus putative basic DNA binding proteins [22] and preliminary studies suggested a role of VP15 in DNA binding using a gel mobility shift assay [29]. However, conclusive experimental evidence for the function of VP15 still needs to be obtained.

DNA-binding- or histone-like proteins are well known from eukaryotic cells where they assist in the packaging and maintenance of DNA into chromosomes. In this process the histones interact and bind to both DNA and to themselves in multimeric structures. Changes in post-translational modifications of such proteins, especially phosphorylation, may be involved in the regulation of gene expression [1, 4, 10]. Little information is known about the functionality of baculovirus basic DNA-binding proteins. Baculovirus DNA-binding proteins are dephosphorylated prior to assembly of the nucleocapsid [7]. In viral genomes in general these basic or histone-like proteins are responsible for condensing viral genomes to facilitate packaging into nucleocapsids [2, 3, 8, 9]. In this report, an indepth analysis is performed on the WSSV basic protein VP15 with regard to its phosphorylation status, protein–protein interactions and DNA-binding properties. We conclude that WSSV VP15 is the histone-like DNA binding protein of WSSV.

Methods

WSSV virus stock and DNA isolation

The virus used in this study originated from infected *Penaeus monodon* shrimp imported from Thailand in 1996 and was obtained as described previously [21]. In short: hemolymph was collected from WSSV-infected *Orconectes limnosis* crayfish and purified on a continuous sucrose gradient (55%–25% w/w). Centrifugation was performed at 80,000 × g for 1.5 h at 4 °C. The band containing the virus was removed from the gradient, sedimented by centrifugation at 30,000 × g and resuspended in TE. Viral DNA was isolated from purified virions as described in Van Hulten et al. [19].

Production and purification of recombinant VP15

For bacterial expression, the entire VP15 ORF was cloned in the pMAL-c2x vector (New England Biolabs) resulting in a N-terminal fusion of VP15 with the maltose binding protein (MBP) of approximately 51 kDa. The entire VP15 ORF was also cloned in the pGEX-2T vector resulting in an N-terminal fusion of VP15 with glutathion-S-transferase (GST) of approximately 33 kDa.

The DNA fragment encoding the entire VP15 ORF (WSSV ORF 109) was amplified from genomic WSSV DNA by PCR using the forward primer 5′-CGGGATCCATGGTTGCCCGAA GCTCC-3′ and reverse primer 5′-TTGCGGCCGCTTAACGCCTTGACTTGC-3′. The

amplified PCR product was ligated in the pGEM-T easy vector (Promega) and sequenced. The ORF was removed from the pGEM-T easy plasmid using the restriction enzyme combinations *Bam*HI, *Pst*I and *Bam*HI, *Eco*RI and ligated into the pMAL-c2x and the pGEX-2T vector, respectively. Overexpression of the fusion proteins was performed in *Escherichia coli* DH5α cells for both constructs. After sonication and centrifugation, the fusion proteins were purified by affinity chromatography using amylose resin (New England Biolabs) for the MBP-VP15 protein and Glutathione Sepharose 4B (Amersham Bioscience) for the GST-VP15 protein according to the manufacturers' protocols. The resulting *E. coli* expressing VP15 and the purified proteins were analyzed by SDS-PAGE and Western analysis; the concentration was determined using the Bradford assay (Bio-Rad).

Phosphorylation of VP15

The phosphorylation status of VP15 was investigated using Western-blots of purified WSSV, infected shrimp tissue, MBP-VP15 overexpressed in bacteria (this paper) and VP15 overexpressed in insect cells [24]. Blots were incubated with a mouse monoclonal antibody (diluted 1:200) directed against phosphoserine, phosphothreonine and phosphotyrosine (Spring Bioscience) to detect phosphorylated proteins. Subsequent incubation with horseradish peroxidase-conjugated rabbit anti-mouse antibodies (1:2000) (Dako) for ECL detection (Amersham Biosciences) of the mouse monoclonals was performed according to the manufacturers' protocols.

Protein–protein interactions: ELISA

An enzyme-linked immunosorbent assay (ELISA) was performed to detect VP15–VP15 interactions. Anti-MBP (New England BioLabs) or anti-GST (Amersham Biosciences) antibodies (100 μl of a 1:5000 dilution in PBS (136 mM NaCl, 2.68 mM KCl, 8 mM Na_2PO_4, 1.76 mM KH_2PO_4 in ddH_2O, pH7.4) containing 0.1% Tween 20) were coated on 96-wells plates for one hour at room temperature. After incubation, the plate was washed two times with tap water, once with distilled water and blocked for three hours at room temperature with 100 μl PBS containing 1% Tween-20 and 2% low-fat milk powder. After incubation and washing, the plate was incubated with a serial dilution of proteins or protein combinations as shown in Table 1 followed by incubation for two hours at room temperature. After washing, the wells were incubated for 1 h at room temperature with anti-MBP antibodies when the plates were first coated with anti-GST or anti-GST if the wells were first coated with anti-MBP (diluted 1:5000 in PBS containing 1% Tween-20). The plates were washed and subsequently incubated for one hour at room temperature with a secondary antibody (goat anti-rabbit when using anti-MBP and swine anti-goat when using anti-GST) conjugated to horseradish peroxidase (diluted 1:5000 in PBS containing 1% Tween-20). After a final wash, the substrate TMB (Fermentas)

Table 1. Set-up of ELISA used for detection of VP15 protein–protein interactions

Antibody coated	Protein mixture	Amount applied per protein (μg)	Detection
α-MBP	MBP-VP15 + GST-VP15	10, 5, 2.5, 1.25, 0.625. . .0.001, 0	α-GST
α-MBP	MBP + GST-VP15 (control)	10, 5, 2.5, 1.25, 0.625. . .0.001, 0	
α-MBP	GST-VP15 (control)	10, 5, 2.5, 1.25, 0.625. . .0.001, 0	
α-GST	GST-VP15 + MBP-VP15	10, 5, 2.5, 1.25, 0.625. . .0.001, 0	α-MBP
α-GST	GST + MBP-VP15 (control)	10, 5, 2.5, 1.25, 0.625. . .0.001, 0	
α-GST	MBP-VP15 (control)	10, 5, 2.5, 1.25, 0.625. . .0.001, 0	

was added and incubated for approximately 20 min. The absorption was measured at 405 nm after stopping the reaction by adding 100 µl 0.2 M sulphuric acid.

Protein–protein interactions: Far-Western

Crude bacterial expressions of MBP-VP15, GST-VP15 and GST alone were separated by SDS-PAGE, electrophoretically transferred to Immobilon-P membranes and gradually renatured at 4 °C in HEPES buffer (HEPES buffer: 20 mM HEPES, pH 7; 50 mM NaCl; 5 mM $MgCl_2$; 1 mM EDTA, 1 mM dithiothreitol (DTT); 10% glycerol) containing 5% low-fat milk powder. The blots were washed and incubated with 100 µg of MBP-VP15 or MBP alone in 5 ml incubation buffer (PBS, 1% Tween-20 and 1% low-fat milk powder) for three hours at room temperature, while rocking gently. The blots were subsequently washed three times 10 min with incubation buffer and incubated for 1 h at room temperature with anti-MBP (1:5000 dilution in PBS-T containing 1% low-fat milk powder). The blots were again washed three times 10 min with incubation buffer and subsequently incubated with horseradish peroxidase-conjugated goat anti-rabbit antibody (1:5000 dilution in PBS-T containing 1% low-fat milk powder) for 1 h at room temperature. After washing three times 10 min with incubation buffer and two times 10 min with PBS, an ECL-detection (Amersham Biosciences) was performed. Using this setup, VP15–VP15 interactions, but also the interaction between the other major WSSV structural proteins (VP28, VP26, VP24 and VP19) was tested.

DNA-binding assay: South-Western

The two VP15 fusion proteins were applied either as a crude bacterial extract or as purified protein and separated in a SDS-PAGE gel and electrophoretically transferred to an immobilon-P membrane. MBP, GST and MBP-VP28 proteins were included as negative controls. After blocking (1.5 hrs at 37 °C in TBS (20 mM Tris-HCl, pH 7.4, 0.15 M NaCl) containing 3% low fat milk powder), the membrane was incubated overnight at 4 °C with 1% blocking reagent (Roche) containing 1.5 µg DIG-labeled DNA per 5 ml. The DNA originated from a PCR product encoding the shrimp phosphoglycerate mutase gene. After incubation, the blot was washed 30 min at RT in TBS-T (TBS containing 0.1% Tween-20) containing 3% ELK followed by washing with TBS-T for 10 min at RT. The DIG labeled probes were detected using anti-digoxigenin-AP (1:5000 dilution in TBS-T containing 0.3% ELK) (Roche) followed by chemiluminescent detection using CSPD substrate (Roche).

DNA-binding assay: Electrophoretic mobility shift assay (EMSA)

The DNA-binding capacity of VP15 was examined in 1% agarose gels in Boric acid buffer (45 mM Boric acid, 45 mM Tris-HCl, pH 8.0). Purified plasmid DNA (pET28a) was mixed with purified VP15 at different ratios in a final volume of 20 µl containing 300 mM $MgCl_2$. This mixture was incubated at 37 °C for 30 min and mixed with 6 × loading buffer (1 mM EDTA (pH 8.0), 50% glycerol in water) before loading on gel. Gels were run for at least three hours at 45 V in Boric acid buffer and the DNA was visualized under UV light after staining the gel in running buffer supplemented with ethidium bromide (0.5 µg/ml) for 30 min.

DNA-binding assay: Dot spot

Spotting DNA and RNA from different sources followed by incubation with purified MBP-VP15 protein was carried out to investigate the DNA/RNA binding properties of native VP15. Different amounts of DNA and/or RNA from different sources were spotted on a Hybond membrane and bound by exposing the spots to UV-light for two minutes (7 mW/cm^2, Fluo-Link transilluminator, Vilber-Lourmat). The membrane was incubated with 40 µg purified MBP-VP15 or 47 µg of purified MBP as a control in DNA binding buffer (0.01 M Tris-HCl,

1 mM DTT, 1 mM EDTA, 0.2 M NaCl, 10% glycerol, 0.4% NP-40, pH8.0) for three hours, while rocking slowly. Subsequently, the membrane was blocked with blocking buffer (3% BSA in TBS) at 37 °C and washed three times 10 min in TBS-T. For detection of the protein, the membrane was incubated with anti-MBP (1:2000 dilution in TBS-T, containing 0.2% ELK) for one hour at room temperature followed by another three washing steps of 10 min and incubation with horseradish peroxidase-conjugated goat anti-rabbit antibody (1:2500 in TBS-T, containing 0.2% ELK) for one hour at room temperature. Finally, the membrane was washed three times followed by ECL detection (Amersham Biosciences).

Results

Production and purification of recombinant VP15

The entire VP15 ORF was successfully overexpressed and purified as an N-terminal fusion with MBP and GST. Bands corresponding to the two fusion proteins were observed at the expected positions of 51 kDa for the MBP and 33 kDa for the GST fusion proteins in a SDS-PAGE gel (Fig. 1). As control proteins, unfused MBP and GST proteins were overexpressed and purified. Bands corresponding to these proteins were also observed at their expected heights of 42.5 kDa for MBP and 26 kDa for GST.

Phosphorylation of VP15

As phosphorylation of DNA-binding proteins may be involved in the regulation of DNA packaging and gene expression, the phosphorylation status of VP15 from different origins was examined. Western blots of VP15 from WSSV infected tissue, purified virions and of recombinant VP15 (insect cell and bacterial expressed) were assayed using antibodies against phosphoserine, phosphothreonine and phosphotyrosine. Phosphorylated proteins (Precision Plus Protein standard,

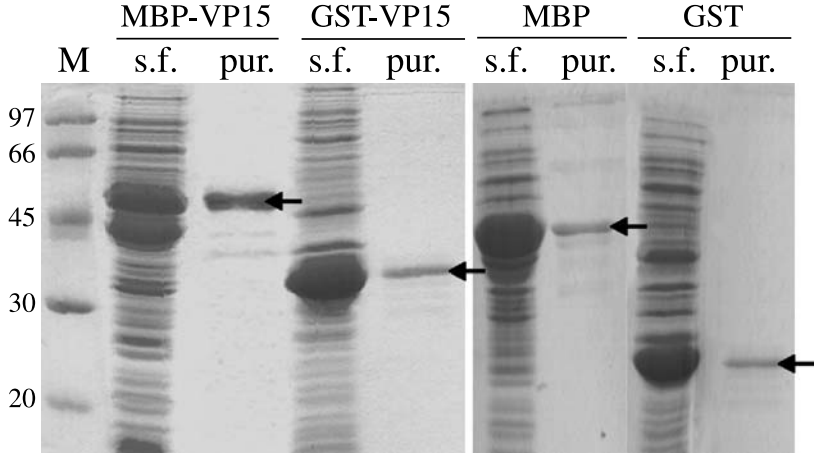

Fig. 1. Coomassie stained SDS-PAGE gels of soluble fractions of *E. coli* expressions (*s.f.*) and purifications (*pur.*) of MBP-VP15, GST-VP15, MBP and GST proteins. Arrows indicate the overexpressed and purified proteins and the numbers next to the marker (*M*) indicate molecular weights in kDa

Bio-Rad) were included in the experiment as positive control. The Western blots showed no positive signals in the samples containing VP15. The positive controls did show a positive signal at the expected heights (data not shown), suggesting that VP15 is not phosphorylated.

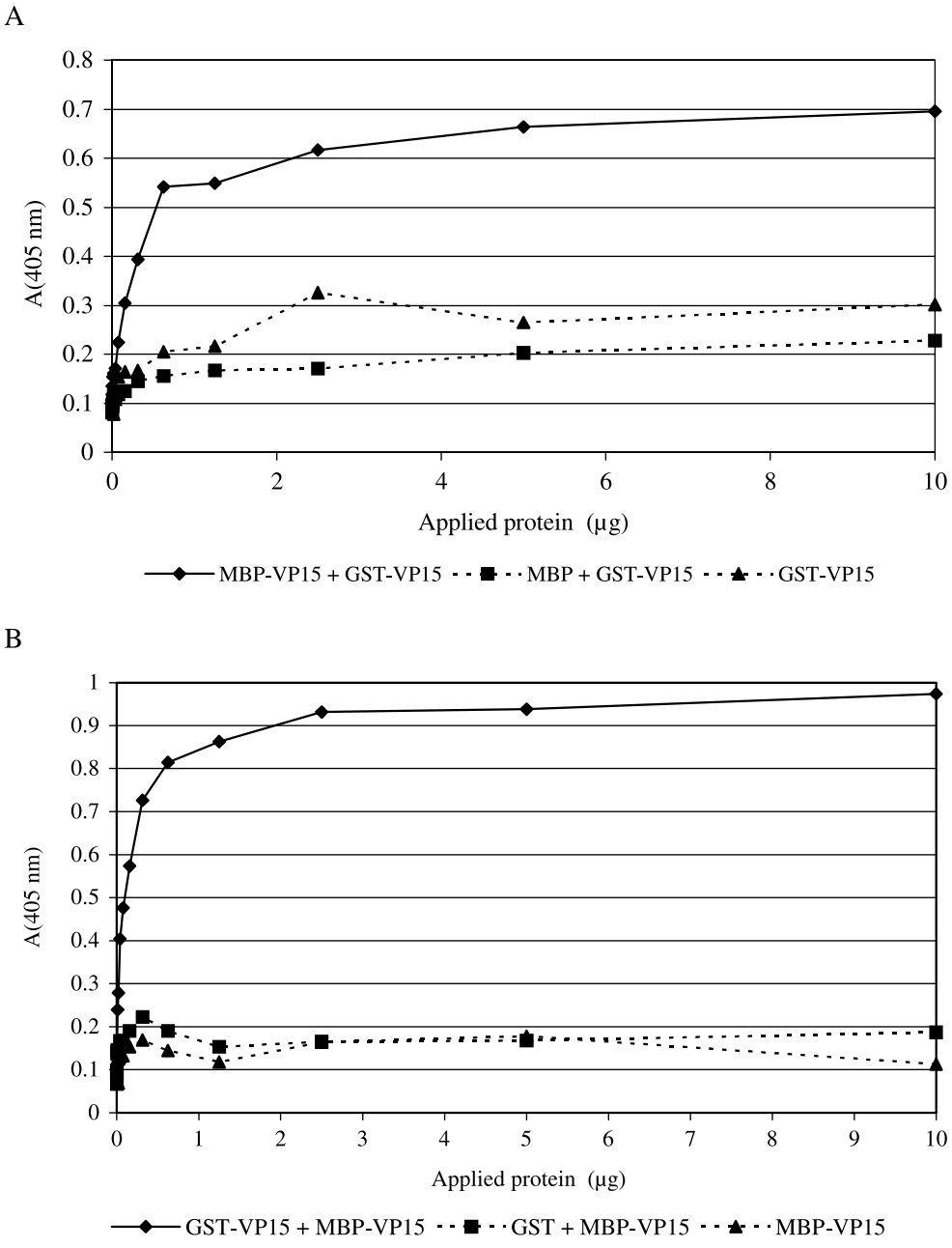

Fig. 2. Results of ELISAs using coated wells with anti-MBP (**A**) and anti-GST (**B**). Protein mixes were applied as indicated in legend. Detection was performed with anti-GST (**A**) and anti-MBP (**B**)

Protein–protein interactions: ELISA

To study the possible interactions between VP15 proteins (homomultimers) ELISA experiments using two differently tagged VP15 fusion proteins (MBP and GST) were performed. In the first experiment wells were coated with anti-MBP followed by incubation with a mixture of GST-VP15 and MBP-VP15 or, as controls, either GST-VP15 alone or GST-VP15 plus MBP. Incubation with this mixture of GST-VP15 and MBP-VP15 resulted in a considerably higher absorbance compared to both controls (Fig. 2a), indicative of a VP15–VP15 interaction. In a second experiment (Fig. 2b), wells were coated with anti-GST and incubated with the same GST-VP15 and MBP-VP15 mixture and either MBP-VP15 alone or MBP-VP15 plus GST as controls. In this case, the difference between the wells incubated with the mixture of GST-VP15 and MBP-VP15 and the controls was even more profound, confirming a VP15–VP15 interaction. ELISA experiments studying the interaction between VP15 and the other four major structural virion proteins (VP28, VP26, VP24 and VP19 expressed as both MBP and GST fusion proteins) failed to show interactions (data not shown).

Protein–protein interactions: Far-Western

To independently confirm the VP15–VP15 interaction observed using ELISA, an overlay assay was performed. MBP-VP15, GST-VP15 and GST alone were separated in a SDS-PAGE gel and transferred to Immobilon-P membranes, renatured and subsequently incubated with either purified MBP-VP15 (Fig. 3a) or purified MBP (Fig. 3b). MBP(-VP15) was detected using MBP-specific antibodies.

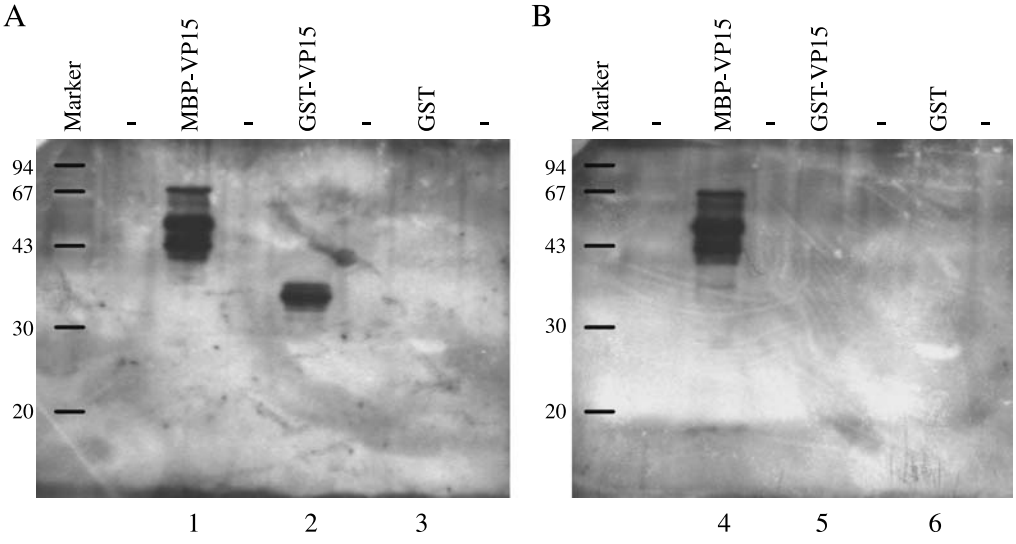

Fig. 3. Far-Western analysis using SDS-PAGE-separated and blotted proteins indicated above the panel. A: MBP-VP15 as overlay protein and anti-MBP to detect MBP-VP15. B: MBP as overlay protein and anti-MBP to detect binding of MBP. Molecular weights in kDa are indicated next to the marker

Figure 3a shows a clear positive signal where GST-VP15 was applied (lane 2), suggesting that there is an interaction between MBP-VP15 and GST-VP15. No signal was observed between GST and MBP-VP15 (lane 3).

To exclude the possibility of an interaction between MBP and VP15, only MBP was used as overlay protein (Fig. 3b). A positive signal was only detected in the control lane with MBP-VP15 (lane 1) and not in GST-VP15 (lane 2). Separate experiments studying the interaction between VP15 and the other four major structural virion proteins (VP28, VP26, VP24 and VP19 expressed as both MBP and GST fusion proteins) did not show specific interactions (data not shown).

DNA-binding assay: South-Western

A South-Western analysis was performed to verify that the DNA binding properties of VP15 previously observed [29] are indeed VP15-specific. For this, a membrane containing SDS-PAGE separated extracts and purified proteins of MBP-VP15, MBP, GST-VP15, GST and MBP-VP28 was incubated with DIG-labeled DNA (Fig. 4). The DNA was consequently detected using CSPD in lanes 1 and 2 containing MBP-VP15, and lanes 4 and 5 containing GST-VP15 fusion proteins at the expected heights. There was no interaction detected in the lanes with MBP, GST and MBP-VP28 (lanes 3, 6 and 7 respectively).

DNA-binding assay: Electrophoretic mobility shift assay (EMSA)

The preference of VP15 for different DNA topologies was investigated using an EMSA experiment. When plasmid DNA is separated in an agarose gel, three major DNA topologies can be distinguished: nicked circle, linear and supercoiled. Different amounts of purified MBP-VP15 were mixed with 500 ng purified plasmid DNA

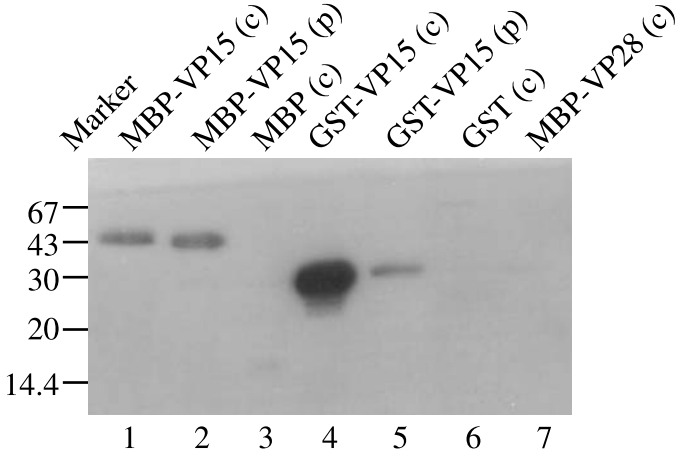

Fig. 4. South-Western analysis of different protein samples; (*c*) crude soluble protein extract, (*p*) purified proteins. The blot was incubated with 2 μg of DIG-labeled DNA followed by CSPD detection. Molecular weights in kDa are indicated next to the marker

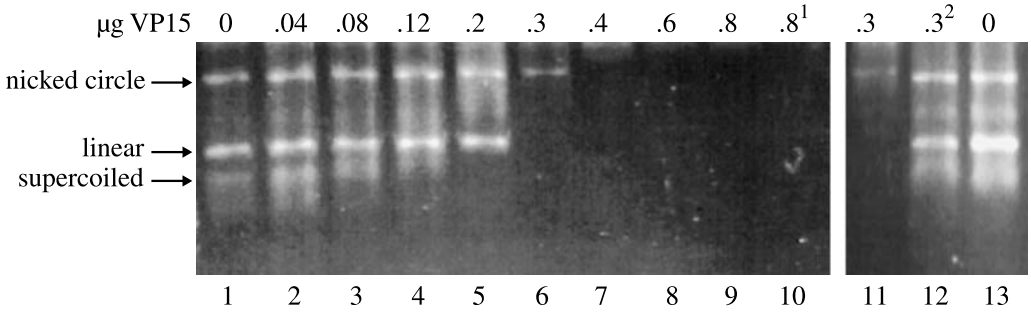

Fig. 5. Electrophoretic mobility shift assay using 0.5 µg of purified plasmid DNA mixed with purified MBP-VP15 prior to loading. The amount of MBP-VP15 protein used in the different mixtures is indicated. As controls no plasmid(1) and purified MBP-VP26(2) were included. The different plasmid topologies are indicated with arrows

(pET28a), incubated and applied onto an agarose gel. Interactions of DNA with proteins results in a retardation or disappearance of the DNA from the separating gel. In Fig. 5 the resulting retardation in mobility is shown. When 0.04–0.12 µg of MBP-VP15 is added, a clear retardation in mobility of only the supercoiled DNA is observed (lanes 2–4). When the amount of MBP-VP15 protein is increased to 0.2 µg, the supercoiled DNA entirely disappears (lane 5). Linearized plasmid DNA disappears at 0.3 µg of MBP-VP15 (lane 6) and ultimately the nicked circle band disappears when 0.4 µg of purified MBP-VP15 is added (lane 7). With an increase in VP15 concentration, the DNA is first retarded and with a further increase of VP15 retained in the loading slot, unable to migrate into the gel. This result suggests there is a topological preference of VP15 to supercoiled DNA over both the linearized and nicked circle topology. When 0.3 µg of purified MBP-VP26 is added, no shift is observed, confirming the specific DNA binding properties of VP15 (lane 12).

DNA-binding assay: Dot spot

For further confirmation of the topological preference of VP15 for supercoiled DNA, and to determine the binding of VP15 to single-stranded DNA, RNA and double-stranded RNA, several Dot-spot assays were performed (Fig. 6).

First, the binding of VP15 to the double-stranded DNA genomes of both WSSV and the baculovirus AcMNPV in their native (circular) and linearized (*Bam*HI digested) form was evaluated. Figure 6a shows that the signal of all circular topologies is similar but considerably higher as compared to the signal observed in their respective linearized forms. When the experiment is repeated with the same DNA, but made single-stranded by heating the DNA (10′ at 95 °C), the signals are similar to the signals found for the linearized DNA. Finally the binding of VP15 to single-stranded and double-stranded RNA was tested but no positive signal was observed, indicating that there is no binding between VP15 and RNA (Fig. 6b). When purified MBP instead of the MBP-VP15 was used in these experiments no positive signals were found (data not shown), confirming that the VP15 protein binds specifically to DNA.

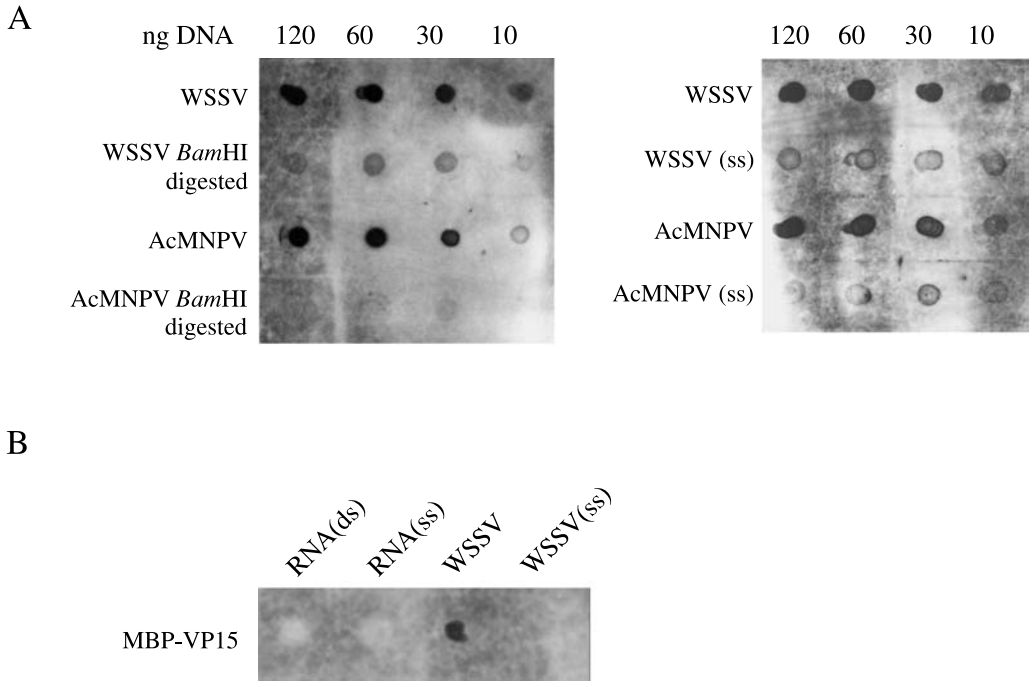

Fig. 6. A: Dot-spot of different treatments of circular DNA, overlaid with MBP-VP15 followed by ECL detection. Indicated are the origin of DNA, treatment and the amount applied to the membrane. **B**: Dot-spot of double-stranded and single-stranded RNA, native WSSV and single-stranded WSSV. Purified MBP-VP15 was used as overlay proteins as indicated and detected by ECL

Discussion

VP15 is one of the three major structural proteins of the WSSV nucleocapsid [24]. Because of similar motifs found in VP15 and several baculovirus DNA-binding proteins, its location in the virion and its predicted physical characteristics, VP15 was assigned to be a DNA binding protein in the WSSV virion [12, 18, 22, 25]. Preliminary results using unpurified bacterial expressions of a VP15-GFP fusion protein in an EMSA suggested that WSSV VP15 is a DNA binding protein [29]. However, direct evidence that the observed retardation was due to binding of VP15 to DNA was not shown.

Although the phosphorylation status of nucleocapsid proteins in baculoviruses may be important for the uncoating process of nucleocapsids [7], no phosphorylated VP15 was detected in WSSV purified virions, WSSV infected tissue and recombinant VP15 from *E. coli* and insect cells.

To further study the function of WSSV VP15, its protein and DNA binding properties were investigated. VP15 was expressed N-terminally fused to MBP and GST. Using these tags, VP15 was readily purified and used in protein–protein interaction and DNA-binding studies. Via ELISA (Fig. 2) and Far-Western experiments (Fig. 3) VP15–VP15 interactions were demonstrated. The formation of VP15 homomultimers might be involved in the formation of nucleocapsids and

although no interaction between VP15 and the four other major structural proteins was observed, it is possible that one or more of the minor WSSV structural proteins [11] participates in this process. Despite the fusion of VP15 to the relatively large MBP and GST tags, the protein appeared to remain functional.

Using the purified VP15 proteins and crude bacterial overexpression lysates, the binding of VP15 to DNA was clearly demonstrated in a South-Western experiment (Fig. 4). As no DNA was detected in association with MBP, GST and MBP-VP28, the binding of DNA to the VP15 fusion proteins must be due to VP15 and not the fused MBP or GST proteins. Since a PCR product of a shrimp gene was used, the binding of VP15 to DNA seemed sequence aspecific. The latter was confirmed in the EMSA and dot-spot experiments (Fig. 6).

More insight in the topological preference of VP15 was obtained in an EMSA (Fig. 5) experiment where a clear preference of VP15 for supercoiled DNA was observed. Supercoiled DNA was the first to show retardation in a serial dilution of VP15. With an increasing amount of VP15 the two other plasmid topologies (nicked circle and linear) also showed retardation, indicating that VP15 has a preference for supercoiled DNA but does not specifically bind to supercoiled DNA. This preference for supercoiled DNA was further substantiated in dot-spot experiments where the binding of VP15 to circular double-stranded DNA molecules was compared to their linearized counterparts (Fig. 6a). In these experiments the sequence a-specificity of VP15 was shown, as the signal found in the WSSV and AcMNPV genome was comparably high. Dot-spot experiments using double-stranded RNA, single-stranded RNA and single-stranded DNA all showed very low signals indicating no or a very low affinity to VP15.

A number of proteins have been described to have a preference to bind supercoiled DNA, which has been referred to as supercoiled-selective (SCS) DNA binding [16]. Because supercoiled DNA contains excess energy which can be released upon binding of proteins, this makes some DNA protein bonds supercoil dependent. Much research has been performed on the human p53 tumor-suppressor protein which plays an important role in the cellular response to DNA damage [15]. This protein binds to supercoiled DNA even in absence of the target sequence in the DNA molecule [17] and exhibits protein interactions as it is most abundantly found as tetramers or multiple tetramers [6]. From a viral perspective the SCS DNA binding of VP15 might be necessary for condensing the large WSSV genome and packaging into the nucleocapsid.

This research has shed further light on the composition of WSSV virions and the function of one of its structural proteins. Future experiments involving VP15 mutants or specific VP15 domains could reveal the important areas for DNA binding and protein–protein interactions. Finally, pseudotyping experiments using recombinant baculoviruses in which the baculovirus DNA-binding protein has been replaced by WSSV VP15, may give more information on the function of VP15.

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References

1. Berger SL (2002) Histone modifications in transcriptional regulation. Curr Opin Genet Dev 12: 142–148
2. Bud HM, Kelly DC (1980) An electron microscope study of partially lysed baculovirus nucleocapsids: the intranucleocapsid packaging of viral DNA. J Ultrastruct Res 73: 361–368
3. Coca-Prados M, Vidali G, Hsu MT (1980) Intracellular forms of simian virus 40 nucleoprotein complexes. III. Study of histone modifications. J Virol 36: 353–360
4. Davie JR, Spencer VA (1999) Control of histone modifications. J Cell Biochem Suppl 32–33: 141–148
5. Durand S, Lightner Donald V, Redman Rita M, Bonami Jean R (1997) Ultrastructure and morphogenesis of white spot syndrome baculovirus (WSSV). Dis Aquat Org 29: 205–211
6. Friedman PN, Chen X, Bargonetti J, Prives C (1993) The p53 protein is an unusually shaped tetramer that binds directly to DNA. Proc Natl Acad Sci USA 90: 3319–3323
7. Funk CJ, Consigli RA (1993) Phosphate cycling on the basic protein of Plodia interpunctella granulosis virus. Virology 193: 396–402
8. Hamatake RK, Aoyama A, Hayashi M (1985) The J gene of bacteriophage phi X174: in vitro analysis of J protein function. J Virol 54: 345–350
9. Hamatake RK, Buckley KJ, Hayashi M (1988) The J gene of phi X174: isolation and characterization of a J gene mutant. Mol Gen Genet 211: 72–77
10. He H, Lehming N (2003) Global effects of histone modifications. Brief Funct Genomic Proteomic 2: 234–243
11. Huang C, Zhang X, Lin Q, Xu X, Hu ZH, Hew CL (2002) Proteomic analysis of shrimp white spot syndrome viral proteins and characterization of a novel envelope protein VP466. Mol Cell Prot 1: 223–231
12. Maeda S, Kamita SG, Kataoka H (1991) The basic DNA-binding protein of Bombyx mori nuclear polyhedrosis virus: the existence of an additional arginine repeat. Virology 180: 807–810
13. Mayo MA (2002) A summary of taxonomic changes recently approved by ICTV. Arch Virol 147: 1655–1663
14. Nadala ECB Jr, Tapay LM, Loh PC (1998) Characterization of a non-occluded baculovirus-like agent pathogenic to penaeid shrimp. Dis Aquat Org 33: 221–229
15. Oren M, Rotter V (1999) Introduction: p53 – the first twenty years. Cell Mol Life Sci 55: 9–11
16. Palecek E, Vlk D, Stankova V, Brazda V, Vojtesek B, Hupp TR, Schaper A, Jovin TM (1997) Tumor suppressor protein p53 binds preferentially to supercoiled DNA. Oncogene 15: 2201–2209
17. Palecek E, Brazdova M, Brazda V, Palecek J, Billova S, Subramaniam V, Jovin TM (2001) Binding of p53 and its core domain to supercoiled DNA. Eur J Biochem 268: 573–581
18. Russell RL, Rohrmann GF (1990) The p6.5 gene region of a nuclear polyhedrosis virus of Orgyia pseudotsugata: DNA sequence and transcriptional analysis of four late genes. J Gen Virol 71: 551–560
19. van Hulten MCW, Westenberg M, Goodall SD, Vlak JM (2000) Identification of two major virion protein genes of white spot syndrome virus of shrimp. Virology 266: 227–236
20. van Hulten MCW, Goldbach RW, Vlak JM (2000) Three functionally diverged major structural proteins of white spot syndrome virus evolved by gene duplication. J Gen Virol 81: 2525–2529

21. van Hulten MCW, Tsai MF, Schipper CA, Lo CF, Kou GH, Vlak JM (2000) Analysis of a genomic segment of white spot syndrome virus of shrimp containing ribonucleotide reductase genes and repeat regions. J Gen Virol 81: 307–316
22. van Hulten MCW, Witteveldt J, Peters S, Kloosterboer N, Tarchini R, Fiers M, Sandbrink H, Lankhorst RK, Vlak JM (2001) The white spot syndrome virus DNA genome sequence. Virology 286: 7–22
23. van Hulten MCW, Witteveldt J, Snippe M, Vlak JM (2001) White spot syndrome virus envelope protein VP28 is involved in the systemic infection of shrimp. Virology 285: 228–233
24. van Hulten MCW, Reijns M, Vermeesch AM, Zandbergen F, Vlak JM (2002) Identification of VP19 and VP15 of white spot syndrome virus (WSSV) and glycosylation status of the WSSV major structural proteins. J Gen Virol 83: 257–265
25. Wilson ME, Mainprize TH, Friesen PD, Miller LK (1987) Location, transcription, and sequence of a baculovirus gene encoding a small arginine-rich polypeptide. J Virol 61: 661–666
26. Witteveldt J, Cifuentes CC, Vlak JM, van Hulten MCW (2004) Protection of Penaeus monodon against white spot syndrome virus by oral vaccination. J Virol 78: 2057–2061
27. Witteveldt J, Vlak JM, van Hulten MCW (2004) Protection of Penaeus monodon against white spot syndrome virus using a WSSV subunit vaccine. Fish Shellfish Immunol 16: 571–579
28. Yang F, He J, Lin X, Li Q, Pan D, Zhang X, Xu X (2001) Complete genome sequence of the shrimp white spot bacilliform virus. J Virol 75: 11811–11820
29. Zhang X, Xu X, Hew CL (2001) The structure and function of a gene encoding a basic peptide from prawn white spot syndrome virus. Virus Res 79: 137–144
30. Zhang X, Huang C, Tang X, Zhuang Y, Hew CL (2004) Identification of structural proteins from shrimp white spot syndrome virus (WSSV) by 2DE-MS. Proteins 55: 229–235

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