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Hordeivirus movement protein encoded by the first gene of the triple gene block (TGB1 protein, TGBp1) interacts in vivo with viral genomic and subgenomic RNAs to form ribonucleoprotein (RNP) particles that are considered to be a form of viral genome (non-virion transport form) capable of cell-to-cell and long-distance transport in infected plants. The structures of these RNPs have not been elucidated. The poa semilatent virus (PSLV) TGBp1 contains a structured C-terminal NTPase/helicase domain and an N-terminal extension region consisting of two domains – a completely intrinsically disordered extreme N-terminal domain and an internal domain (ID) with structure resembling a partially disordered molten globule. Here, we characterized the structures assembled in vitro by the full-length PSLV TGBp1 alone or in the presence of viral RNA. The PSLV TGBp1 was capable of multimerization and self-assembly into extended high-molecular-mass complexes. These complexes disassembled to apparent monomers upon incubation with ATP. Upon incubation with viral RNA, the PSLV TGBp1 in vitro formed RNP structures that appeared as filamentous particles resembling virions of helical filamentous plant viruses in morphology and dimensions. By comparing the biophysical characteristics of PSLV TGBp1 and its domains in the presence and absence of RNA, we show that the ID plays the main structural role in the self-interactions and RNA interactions of TGBp1 leading to the assembly of virus-like RNP particles.

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INTRODUCTION

Barley stripe mosaic virus (BSMV) and Poa semilatent virus (PSLV), representatives of the genus Hordeivirus, have rod-shaped particles with helical structures (Jackson et al., 2009). The BSMV and PSLV genomes consist of three positive-sense ssRNAs designated RNAx, RNAβ and RNAγ. RNAx and RNAγ encode two replicate subunits and a small cysteine-rich regulatory γ/b protein. RNAβ encodes a coat protein (CP) and triple gene block (TGB) of movement proteins (MPs) TGBp1, TGBp2 and TGBp3 (Morozov & Solovyev, 2003; Jackson et al., 2009). Many plant viruses, including those encoding TGB, need CP for cell-to-cell and long-distance virus transport in infected plants. However, in the case of hordeiviruses these processes do not require CP. The ability of BSMV CP-deficient mutants to establish systemic infection provides strong evidence that virus movement occurs via a ribonucleoprotein (RNP) complex (Petty & Jackson, 1990; Lim et al., 2008). Brakke et al. (1988) isolated a RNP complex from BSMV-infected barley plants and showed that it contains a protein similar in size to TGBp1. Detailed biochemical analyses of the BSMV RNP complex from infected plants demonstrated that TGBp1 is the major, if not the only, virus protein that forms RNP complexes with viral genomic and subgenomic RNAs (Donald et al., 1997; Lim et al., 2008; Jackson et al., 2009). Such TGBp1–RNA RNPs are considered to be a form of viral genome capable of cell-to-cell and long-distance transport in plants (non-virion transport form) (Morozov & Solovyev, 2003; Lim et al., 2008). Small membrane TGBp2 and TGBp3 proteins are necessary for intracellular transport of RNP complexes to plasmodesmata of cell walls (Morozov & Solovyev, 2003; Jackson et al., 2009, Verchot-Lubicz et al., 2010).

The biochemical and functional properties of hordeivirus TGBp1s are well studied. Hordeivirus TGBp1 contains a
C-terminal NTPase/helicase domain (HELD) with seven conserved motifs of superfamily 1 NTPases/helicases (Gorbunova & Koonin, 1993). This domain exhibits in vitro NTPase and RNA helicase activities, and binds to RNA cooperatively but non-specifically (Bleykasten et al., 1996; Donald et al., 1997; Kalinina et al., 2001, 2002; Leshchiner et al., 2006). Mutations in any of the conserved HELD motifs block viral cell-to-cell movement (Donald et al., 1997). Additionally, the hordeivirus TGBp1s have a long N-terminal extension region preceding the NTPase/helicase domain that is variable in sequence and length with multiple RNA-binding sites (Donald et al., 1997; Kalinina et al., 2001; Morozov & Solovyev, 2003; Makarov et al., 2009). Combining several disorder prediction methods and experimental approaches, it was demonstrated that this region of the PSLV TGBp1 (referred to as N63K in our previous work; Kalinina et al., 2001) has large intrinsically disordered areas and consists of two domains - a completely intrinsically disordered extreme N-terminal domain (NTD) (aa 1–190) and an internal domain (ID) (aa 200–290) with structure resembling a partially disordered molten globule (Makarov et al., 2009, 2012). Both domains demonstrate the ability for non-specific RNA binding; NTD interacts with RNA non-cooperatively and ID interacts cooperatively (Makarov et al., 2009); the whole N63K binds to RNA in a non-cooperative manner (Kalinina et al., 2001). The possible functional significance of RNA binding was revealed by mutagenesis of the PSLV TGBp1 extension region. Mutations that disrupted either of two clusters of positively charged amino acids responsible for RNA binding blocked long-distance, but not cell-to-cell movement of viral infection (Kalinina et al., 2001). Interestingly, these clusters are responsible for other functions in addition to RNA-binding activity: they are indispensable for nuclear/nucleolar targeting of PSLV TGBp1, and contain nuclear and nucleolar localization signals and sites of interaction with coilin, a main structural protein of Cajal bodies with numerous activities (Semashko et al., 2012a, b). N63K is also able to bind in vivo and in vitro another nuclear protein – fibrillarin (Semashko et al., 2012a). Recent studies revealed that nuclear/nucleolar localization of viral protein is an indispensable phase for long-distance transport of many plant viruses (Taliansky et al., 2010; Solovyev & Savenkov, 2014).

Despite numerous data about the biological functions and biochemical activities of hordeivirus TGBp1s, there is almost no information concerning their structural features. The structure and formation mechanism of a non-virion transport form (RNP complex) by TGBp1 remain unclear. In our previous works (Makarov et al., 2010, 2012), we showed that the N-terminal extension region and the ID of PSLV TGBp1 can form in vitro multimeric filamentous structures. In this study, we demonstrate that the full-length PSLV TGBp1 is also able to form similar high-molecular-mass complexes. Moreover, in the presence of RNA, PSLV TGBp1 forms RNP complexes resembling filamentous virions of helical plant viruses. Based on the in vitro biophysical properties of full-length PSLV TGBp1 and its domains, we propose that the ID provides a key structural feature in the process of non-virion RNP assembly.

RESULTS

Recombinant full-length PSLV TGBp1 (63K protein) forms multimers in solution

Dynamic laser light scattering (DLS) is used for estimation of the size distribution of individual protein molecules and protein complexes in solution (Barilla et al., 2005; Nemykh et al., 2008). Results are represented as the number (percentage) of certain size particles, where the particle size is its hydrodynamic diameter approximated to globular particle parameters or as the volume (percentage) occupied by particles of this size. The recombinant PSLV TGBp1 with molecular mass 63 kDa (also referred to as the 63K protein) forms two types of complexes in solution at 25 °C – more abundant particles with a mean size of 35 ± 15 nm (the sizes of these complexes vary from one experiment to another and for some preparations they reach ~50 nm) and particles with a hydrodynamic diameter of 300 ± 150 nm (Fig. 1a, b). Despite the fact the large particles occupy a substantial volume (Fig. 1a, solid line), their content (number) is very small (~1.0 %). Therefore, the peak corresponding to large particles is absent on the curve of particle distribution by number (Fig. 1b, solid line). Complexes with similar parameters and distribution were formed at 25 °C by the isolated ID and N63K, whereas the mean diameter of the NTD particles was ~15 ± 3 nm (Makarov et al., 2009, 2012). These complexes are formed spontaneously and are apparently unstable. The addition of 0.01 % SDS not inducing formation of detergent micelles results in the conversion of the complexes into particles of 10.0 ± 2.0 nm (Fig. 1a, b). These particles are probably low-molecular-mass oligomers. An important property of the 63K protein is the dependence of the complex sizes and their ratio in preparations on the incubation temperature. When the temperature was increased to 37 °C, large complexes disappeared completely and particles of 2–4 nm prevailed (Fig. 1a, b). We propose that these particles are protein monomers. In contrast, decreasing the temperature to 4 °C was accompanied by protein aggregation and in this case complexes of 200–500 nm became predominant. This effect can be explained by the phenomenon of ‘cold denaturation’ for proteins with loose tertiary structure – in the course of cooling, hydrophobic intramolecular interactions loosen, which leads to ‘disintegration’ of the tertiary structure of protein molecules with their subsequent aggregation due to intermolecular interactions (Privalov, 1990). Both N63K and ID, but not NTD, demonstrated similar properties (Makarov et al., 2010, 2012). Thus, ID is apparently the domain determining the ability of PSLV 63K protein for self-interaction.
Disassembly of the 63K protein multimers in the presence of ATP

The PSLV 63K protein displays NTPase and RNA helicase activity \textit{in vitro} (Kalinina et al., 2002). We studied the effect of ATP on the multimerization of 63K protein by DLS. Fig. 2 presents the particle size distribution upon incubation of the 63K protein preparation at 25°C with 5 mM ATP and 5 mM MgCl₂. The complexes formed at 25°C demonstrate ‘standard’ distribution – abundant complexes with mean sizes of 55 ± 15 nm and fewer large complexes with diameters of 300 ± 150 nm. Incubation for 10 min resulted in a remarkable decrease in the particle size from ~55 nm to 3.0 ± 1.0 nm, which indicated disassembly of multimeric complexes to apparent monomers. After 20 min incubation, the mean size of the particles increased to ~25 ± 10 nm. During further incubation (30–40 min), the particle sizes and their distribution gradually returned to the initial state (multimeric 63K protein complexes identified in the first stage), which might be due to the depletion of ATP in the sample. The observed disintegration effect might be related to changes in the structure of protein molecules caused by the activation of the enzymic HELD (aa 321–541) in the presence of ATP.

Interaction with RNA induces structural transitions in the 63K protein

Additional data favouring the participation of the ID in the multimerization/self-interaction of the 63K protein \textit{in vitro} were obtained from studying the changes in the 63K protein structure caused by its interaction with RNA. To determine the secondary structure of the full-length PSLV 63K protein, circular dichroism (CD) spectra of the recombinant protein in the far-UV (190–250 nm) region were measured. Remarkably, the 63K protein possessed a CD spectrum characteristic of polypeptides with a significant content of intrinsically disordered structures and with molar ellipticity at a minimum at 203 nm [h]203 of about −5500° (Fig. 3a).

We studied the influence of RNA on the secondary structure of the 63K protein. In these experiments, tRNA was used to minimize the contribution of RNA to the molar ellipticity measured in the far-UV region. The addition of tRNA to the 63K protein preparation at molar protein : RNA ratios of 50 : 1 and 100 : 1 resulted in a significant change in the CD spectra of the protein (Fig. 3a). The peak at 203 nm decreased, which was characteristic of transition of a portion of protein molecules from the disordered to the ordered state. Simultaneously, the intensity of the positive peak at ~220 nm increased, pointing to an increase in the β-structure content. Fig. 3(a) demonstrates that the initial changes in the CD spectrum of the 63K protein were observed already at a molar protein : RNA ratio of 200 : 1. Significantly, similar changes were found in the CD spectrum of ID in the presence of tRNA (molar protein : RNA ratio of 50 : 1) (Fig. 3b). The addition of tRNA at the same molar ratio to NTD and N63K did not lead to changes in their secondary structure (Fig. 3c, d).

Taking into account that the contribution of the tRNA content to the molar ellipticity change is insignificant and can be neglected, all the changes in the spectra can be attributed to changes in the structure of the protein molecules. These changes induce protein–protein interactions followed by multimerization of the 63K protein. A low concentration of RNA might be a primer initiating formation of multimer complexes as suggested earlier in the case of the PSLV ID (Makarov et al., 2010). Indeed, our
data indicate that the addition of tRNA at low concentrations to the 63K protein is accompanied by its multimerization into large 200–300 nm aggregates, as indicated by DLS (Fig. S1, available in the online Supplementary Material). The observed structural changes are obviously determined by changes in the structure of the ID in the content of the full-length protein.

Transmission electron microscopy (TEM) visualization of structures formed by the 63K protein and its complexes with RNA

TEM was used to visualize structures formed by the full-length 63K protein. Fig. 4(a) shows topographic images of the 63K protein preparation obtained using a JEOL JEM-1011 electron microscope. The preparation contained a heterogeneous set of filamentous structures with diameters of ~20–30 nm, variable in length from short (300–500 nm) to long (up to 2 μm). Fig. 4(b) shows these structures at higher magnification. The observed filaments can branch and form assemblies. In addition, Fig. 4(b) also shows globules of various sizes (15–30 nm). The extended filamentous structures formed by the 63K protein were also visualized by atomic force microscopy (AFM). These structures resemble those referred to as 'beads-on-string' structures (Fig. S2). Elongated filamentous structures of a similar type were observed in preparations of recombinant proteins comprising ID and N63K (Makarov et al., 2010).

In our previous work, we showed that the PSLV 63K protein interacts with tobacco mosaic virus (TMV) RNA forming protein–RNA complexes, which were analysed by the retardation method in non-denaturing agarose gels (Kalinina et al., 2001). The non-specific manner of the protein–RNA interaction allowed us to use TMV RNA to obtain RNP complexes in our experiments. The PSLV 63K protein was mixed with TMV RNA at a molar protein : RNA ratio of 100 : 1 and incubated for 30 min at 25 °C. This molar ratio was chosen for several reasons: (i) this ratio was saturating for the formation of protein–RNA complex analysed by gel shift (the protein cooperatively bound to all RNA molecules in the sample; Kalinina et al., 2001), (ii) as shown above, at this ratio the maximum change in the secondary structure of the protein was revealed, and (iii) at this ratio the protein almost completely inhibited RNA translation in a cell-free protein-synthesizing system (Fig. S3) in conditions described in Rodionova et al., (2003). In an electron micrograph (Fig. 4c) of the PSLV 63K protein–TMV RNA preparation along with protein complexes of different sizes, which are likely induced in

![Fig. 2. Disassembly of 63K protein multimers during incubation with ATP. Size distribution of particles by volume measured by DLS during incubation of the PSLV 63K protein preparation with 5 mM ATP and 5 mM MgCl₂ at 25 °C.](http://jgv.microbiologyresearch.org)
the presence of RNA, we observed extended filamentous virus-like particles. The assembled filamentous particles had lengths of up to 300–350 nm and widths of ~13 nm. These PSLV TGBp1–RNA RNP particles demonstrated a clearly visible central channel and regions of uniform helical structure. In terms of morphology and dimensions, the RNP structures resembled filamentous virions of helical plant viruses, such as virions of potato virus X with a particle width of 13.5 nm.

**DISCUSSION**

Based on the results of numerous studies, mainly on BSMV as the type member of the genus *Hordeivirus*, it has been proposed that the RNP complex is the primary determinant for transport of hordeivirus RNAs during local and systemic virus invasion of plants (Lim *et al.*, 2008; Jackson *et al.*, 2009; Verchot-Lubicz *et al.*, 2010). It was suggested that TGBp1 functions directly to bind the individual BSMV RNAs *in planta*, forming separate nucleocapsids (Lim *et al.*, 2008). Nevertheless, the exact structure of TGBp1–RNA complexes was not known.

Here, we demonstrate that *in vitro* incubation of PSLV TGBp1 and TMV RNA leads to assembly of filamentous RNP particles resembling filamentous virions of helical plant viruses in terms of morphology and dimensions. Thus, we provide direct evidence that PSLV TGBp1 alone is able to encapsidate RNA, forming virus-like nucleocapsids with a presumably helical structure.

To study the formation of non-virion PSLV RNP *in vitro*, we investigated in detail the biophysical properties of the full-length PSLV TGBp1 and compared them with the characteristics of individual TGBp1 domains (NTD, ID...
and HELD) obtained previously (Makarov et al., 2009, 2010, 2012). To assemble RNP particles, the TGBp1 has to be engaged in homologous protein–protein and protein–RNA interactions. All three domains of the hordeivirus TGBp1s participate in homologous binding interactions and have RNA-binding activities (Donald et al., 1997; Kalinina et al., 2001; Leshchiner et al., 2006; Lim et al., 2008; Makarov et al., 2009). Here, using DLS and TEM, we demonstrate that the full-length PSLV 63K protein is capable of self-assembly into extended filamentous structures and forms irregular oligomers/multimers of different orders. Remarkably, this protein possesses a CD spectrum characteristic of polypeptides with a significant content of intrinsic disorder in spite of the presence of mainly ordered HELD. The loose tertiary structure of the protein is confirmed by its ability to aggregate at lower temperatures or under treatment with low concentrations of SDS. Similar properties were revealed by the N-terminal region of PSLV TGBp1 (N63K) and its ID (Makarov et al., 2010, 2012). These data indicate that the ID is the structural domain determining the ability of PSLV TGBp1 for multimerization/self-assembly.

The PSLV TGBp1 shares these properties with intrinsically disordered viral nucleoproteins forming internal nucleocapsids of a number of animal viruses with a negative-stranded RNA genome or with CPs of helical plant viruses (Namba, 2001; Longhi et al., 2003; Ivanyi-Nagy et al., 2008; Uversky & Longhi, 2012). For example, the nucleoproteins of influenza and rabies viruses are a well-studied class of viral proteins that form extended filamentous structures (Ruigrok & Baudin, 1995; Iseni et al., 1998). In the case of plant viruses, the ability to form virus-like particles that do not contain RNA has long been known for CPs

**Fig. 4.** Structures assembled in vitro by the 63K protein alone and in the presence of TMV RNA. (a, b) Electron micrographs of the 63K protein complexes staining with 2 % uranyl acetate [bar, 1 µm (a), 500 nm (b)]. (c) The 63K protein–TMV RNA particles stained with 2 % uranyl acetate (bar, 500 nm). Inset, image of a particle shown at higher magnification (bar, 100 nm).
of rod-shaped tobatoviruses (Diaz-Avalos & Caspar, 1998) as well as for CPs of flexible filamentous poty- and potexviruses (Anindya & Savithri, 2003; Tremblay et al., 2006).

It was shown that hordeivirus TGBp1s have multiple RNA-binding sites (Donald et al., 1997; Kalinina et al., 2001; Leshchiner et al., 2006; Makarov et al., 2009). We studied the influence of RNA binding on the PSLV TGBp1 secondary structure. Noticeable changes of the TGBp1 CD spectrum are observed after addition of tRNA, which is characteristic of increasing β-structure element content. Importantly, similar conversion in the presence of tRNA was found in the CD spectrum of ID, which binds RNA cooperatively (Makarov et al., 2009). Under the same conditions, obvious changes in the CD spectra of other RNA-binding domains, i.e. NTD, N63K and HELD, were not revealed. Taking into account that the tRNA concentration is negligible, we suppose that the transitions of the TGBp1 secondary structure are initiated by tRNA as a primer and followed by interactions between protein molecules. The data indicate a principal contribution of ID to homologous protein–protein and protein–RNA interactions.

The incubation of PSLV TGBp1 with TMV RNA leads to assembly of filamentous RNP particles of various lengths up to 300–350 nm and a width of ~13 nm with elements of helical structure. In summary, these data indicate that TGBp1 is able to form RNP particles with a regular structure and the ID performs a major structural role in the process of RNP assembly. Previously, we showed that the ID structure might be significantly unstable (Makarov et al., 2012); therefore, we believe that based on RNA interactions, the ID secondary structure conversion from mainly disordered to ordered and rich in β-structure elements is an essential step for realization of the structural functions of the ID. Thus, RNA–protein interactions are directly involved in the assembly RNP particles along with protein–protein interactions.

The domains/proteins with a significant amount of β-structure can effectively interact with nucleic acids and bind homologous partners through the formation of inter-molecular cross-β structures (Murzin, 1993; Naveed & Liang, 2014). All-β-fold is typical for proteins forming RNP particles, in particular for the cold shock domain with a β-barrel structure of YB-1 protein, the major protein of cellular messenger RNP complexes (Skabkin et al., 2004), and probably for the central core of MPs of plant viruses, which belong to the 30K superfamily (Mushegian & Elena, 2015).

Data on the possible structure of non-virion RNP are limited to a few examples that describe the structures assembled in vitro from viral RNA and MPs of several plant viruses. In general, two main types of structures were observed depending on the protein: RNA ratio and the ability of MP to interact with RNA cooperatively or non-cooperatively: ‘beads-on-string’ structures, which represent individual MP molecules bound to RNA non-cooperatively and separated by protein-free RNA segments of varying length, and strands of RNA molecules tightly and cooperatively bound by clusters of MP molecules, without any apparent protein-free RNA segments (Kiselyova et al., 2001; Nurkiyanova et al., 2001; Kim et al., 2004). The aggregated state was often shown for the latter type of complexes (Kiselyova et al., 2001; Nurkiyanova et al., 2001). In our previous work, using AFM, we described complexes of TMV RNA and the N-terminal region (N63K) of the PSLV 63K protein as partially condensed threads with elements of a ‘beads-on-string’ structure and PSLV 63K–TMV RNA complexes as large globular multimer structures with a halo of thin threads protruding from them (Kalinina et al., 2001). The first filamentous RNP particles were found and isolated from plants infected with a TMV-30B vector expressing the umbravirus ORF3 protein (functioning as a long-distance MP) in place of TMV CP (Taliansky et al., 2003). These particles with a diameter of 13–14 nm had elements of helical structure, resembling virus-specific filamentous particles, but were not as uniform as classical virions. Similar RNP complexes were assembled in vitro from umbravirus RNA, ORF3 protein and fibrillarin (Kim et al., 2007). Virus-like RNP complexes formed by the PSLV TGBp1 protein and TMV RNA in vitro are comparable with umbravirus RNP particles in terms of morphology and structure.

Based on the present results and previously obtained data, we propose a model for hordeivirus non-virion RNP assembly. Assembly of RNP complexes is initiated by direct binding of the extreme NTD to viral RNA, causing structural changes in the TGBp1 ID that are accompanied by homologous protein–protein interactions. Further homologous protein–protein interactions and cooperative binding of protein molecules (mainly the ID domain) to RNA likely occur simultaneously. We could not exclude that along with ID, an adjacent HELD subdomain might participate in this process, presumably forming a pocket responsible for RNA binding and oligomerization. As shown previously, the N-terminal subdomain of HELD takes part in cooperative RNA binding, homologous protein interactions and ATPase activity (Leshchiner et al., 2006; Lim et al., 2008). The NTD and a C-terminal subdomain of HELD are apparently located on the surface of RNP particles, and this assumption agrees well with functional properties of TGBp1s. The assembled TGBp1–RNA complex is non-translatable and might be considered as a non-virion transport form of hordeiviral RNA facilitating both cell-to-cell and long-distance virus transport. The RNP complex could be activated by destabilization of filamentous particles during interaction of exposed regions with cellular or viral partners. Reported interactions between ID/NTD and ID/HELD (Makarov et al., 2009) and NTPase/helicase activities of HELD (Kalinina et al., 2002; Leshchiner et al., 2006) might be the basis for
remodelling and activation of the RNP complexes formed by TGBp1 at different phases of virus movement. The ability to attenuate the homologous protein–protein interactions was detected in our experiments on disassembly of TGBp1 multimers to monomers under incubation with ATP.

Results obtained in vitro are in good agreement with the biological properties of viruses with hordei-like TGBs containing N-terminal extension regions in the content of TGBp1s. In the case of a short NTD, these viruses use RNPs as transport forms only for cell-to-cell movement. For long-distance transport, they need CPs and possibly virions (Morozov & Solovyev, 2003). In hordeiviruses and pomoviruses with long N-terminal extension regions, CPs are dispensable for both cell-to-cell and systemic movement (Brakke et al., 1988; Petty & Jackson, 1990; Savenkov et al., 2003; Lim et al., 2008; Jackson et al., 2009; Torrance et al., 2009) with only one exception: the potato mop-top virus (PMTV) genomic RNA component encoding CP and CP-readthrough moves systemically in the form of a modified virus particle (Torrance et al., 2009). These data support our hypothesis that a partially disordered ID directly participates in formation of the RNP complex, which is obviously essential for both virus cell-to-cell and long-distance movement (in representatives of the genera Hordeivirus and Pomovirus). Mutations disrupting the structure or function of the ID abolish virus movement (Donald et al., 1997; Solovyev et al., 1999). The recent data of Lukhovitskaya et al. (2015) provide additional evidence in favour of our suggestion. They indicated that ID is required for PMTV TGBp1 self-interaction and cell-to-cell movement. NTD deletion is dispensable for these functions. At the same time, PMTV NTD is required for importin-α interaction in plants, nucleolar targeting and long-distance movement (Wright et al., 2010; Lukhovitskaya et al., 2015). In our previous works, we also demonstrated that PSLV NTD is needed for long-distance movement (Kalinitina et al., 2001), localization in the nucleus/nucleolus and interaction with two nuclear proteins, i.e. fibrillarin and coilin (Semashko et al., 2012a, b). Thus, the unfolded NTD presumably located on the outer surface of the RNP particle could not only stabilize/protect genomic RNAs, but also fulfil numerous functions, binding to distinct ligands at different stages of virus infection.

**METHODS**

Expression of recombinant protein genes in *Escherichia coli* cells and purification of His₆ recombinant proteins by affinity chromatography on nickel-nitrilotriacetic acid (Ni-NTA) agarose. The previously obtained recombinant plasmids (Kalinitina et al., 2001; Makarov et al., 2009) were used to transform M15 *E. coli* cells containing the high-copy repressor plasmid pRep-4. Clones expressing recombinant protein genes corresponding to the full-length 63K protein, its N-terminal half (N63K), NTD and ID were grown overnight at 37 °C on standard 2 × YT medium in the presence of ampicillin (100 µg ml⁻¹) and kanamycin (25 µg ml⁻¹). The overnight culture was diluted 10-fold and grown at 37 °C to A₆₀₀ 0.8. Protein expression was induced by addition of IPTG (final concentration 1–2 mM) for 2–4 h. Cells were pelleted at 6000 r.p.m. for 10 min in a J-21 centrifuge (Beckman). The recombinant proteins, fused at the N terminus with His₆-tag, were purified on Ni-NTA agarose in accordance with the Qiagen isolation protocol under denaturing conditions.

DLS. Recombinant protein preparations were dialysed against 1 mM HEPES buffer, pH 7.0, and analysed using DLS. Measurements were carried out using a Zetasizer Nano ZS device (Malvern Instruments) with a He/Ne laser (633 nm, 10 mW) as the light source. Temperature of samples was maintained within 0.1 °C using a Peltier thermostating system. Light scattering was measured at an angle of 173°. Usual polystyrene cells with a 10 mm optical path were used for the experiments. The sample volume in a cell was 1 ml. Measurements were carried out in 1 mM HEPES buffer, pH 7.0, in the protein concentration range 0.10–0.15 mg ml⁻¹. Processing of autocorrelation functions and determination of the percentage of particles of a certain size or volume occupied by particles of this size were performed using Dispersion Technology Software version 5.10.

CD spectroscopy. Protein samples at a concentration of 100 µg ml⁻¹ in 1 mM HEPES buffer, pH 7.0, were loaded into 1–2 mm cells and CD spectra were recorded from 185 to 250 nm at 25 °C in a Chirascan CD spectrometer (Applied Photophysics). The CD spectra were recorded at rate 0.5–1.0 nm s⁻¹ with baseline subtraction. The measured spectra were smoothed using the instrument’s software. The molar ellipticity [θ] value calculations were based on a mean amino acid residue molecular mass of 110 Da.

TEM. The PSLV 63K protein was mixed with TMV RNA at a molar protein : RNA ratio of 100 : 1 and incubated for 30 min at 25 °C. The sample was centrifuged at 10 000 g for 10 min, diluted to concentrations of ~10–50 µg ml⁻¹, applied to 200-mesh carbon-coated copper electron microscope grids and left to settle for 1–2 min. Samples for electron microscopy were prepared using the standard method of negative staining with 2 % (w/v) uranyl acetate solution (pH 6.0) for 2 min, and examined using a JEOL JEM-1011 electron microscope with a Gatan ES 500W Erlangshen digital camera and Digital Micrograph 1.85 software.

AFM. The protein solution was allowed to adsorb onto the surface of freshly cleaved mica for 1 min, after which the solution was carefully removed with filter paper. The substrate was immediately placed on to a drop of double-distilled Millipore water (this procedure was repeated twice) and the surface was dried under airflow. This sample preparation method was used to eliminate any remaining salts and minimize artefactual aggregation during drying. AFM analysis was performed on these samples using a NanoScope IIIa microscope (Digital Instruments) operating in tapping mode with a typical scan rate of 1 Hz. The measurements were performed in air in tapping mode using sharp silicon cantilevers (NT-MDT) with a guaranteed tip radius of 10 nm.

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