Potato virus Y (PVY) is a common potyvirus of agricultural importance, belonging to the picornavirus superfamily of RNA plus-stranded viruses. A covalently linked virus-encoded protein VPg required for virus infectivity is situated at the 5′ end of potyvirus RNA. VPg seems to be involved in multiple interactions, both with other viral products and host proteins. VPgs of potyviruses have no known homologs, and there is no atomic structure available. To understand the molecular basis of VPg multifunctionality, we have analyzed structural features of VPg from PVY using structure prediction programs, functional assays, and biochemical and biophysical analyses. Structure predictions suggest that VPg exists in a natively unfolded conformation. In contrast with ordered proteins, PVY VPg is not denatured by elevated temperatures, has sedimentation values incompatible with a compact globular form, and shows a CD spectrum of a highly disordered protein, and HET-HELSOFAST NMR analysis suggests the presence of large unstructured regions. Although VPg has a propensity to form dimers, no functional differences were seen between the monomer and dimer. These data strongly suggest that the VPg of PVY should be classified among intrinsically disordered proteins. Intrinsic disorder lies at the basis of VPg multifunctionality, which is necessary for virus survival in the host.

It is generally accepted that a protein should attain stable folded conformation to perform its specific physiological functions. However, we are beginning to realize that many proteins contain large unfolded regions, and there are examples of entire proteins lacking regular secondary and tertiary structures (1). There is abundant evidence that the unstructured state, common to all living organisms, is essential for basic cellular functions. Approximately 10% of proteins are predicted to be fully disordered (2). Proteins should fulfill multiple functions through interactions with distinct partners, which requires structured proteins to use separate binding surfaces or domains. In contrast, intrinsically unstructured proteins can, because of their malleability, use the same regions or overlapping surfaces for different interactions by taking advantage of their capacity to adopt different conformations upon binding.

Potyvirus VPg is a virus-coded terminal protein of ~22 kDa, attached to the 5′ end of virus RNA genome. During the virus life cycle VPg is expressed as a part of a larger polypeptide, which self-processes into mature viral protein components. VPg is the N-terminal part of one of the first products, VPg-Pro (also called NIa), liberated by polyprotein proteolysis. At the end of the viral cycle VPg is attached to the 5′ terminus of the progeny genome and is packaged into virions along with viral RNA.

VPg is required for potyvirus infectivity. The latter is abolished when genomic PVY RNA is treated with proteinase K (3). Moreover, mutation of the tyrosine residue involved in the linkage between RNA and the VPg protein is lethal for virus growth and replication (4, 5). VPg seems to be involved in multiple interactions, with both other viral products and host proteins. It has been implicated in translation (6), long distance movement in plant tissue (7), and replication (8). It has also been shown to interact with viral RNA polymerase, suggesting a role, possibly as a primer, in viral RNA synthesis (8–10). VPg, as part of the VPg-Pro protein, has been observed to translocate to the host cell nucleus at the beginning of infection, possibly by an importin-dependent mechanism (11–14). Indeed, VPg contains a two-partite nuclear localization signal (amino acid residues 1–11 and 43–57 in VPg of tobacco etch virus potyvirus (12)). However, the role of VPg in the nucleus is not clear because mature potyvirus particles accumulate in the cytoplasm of infected plants. Nevertheless, knocking out VPg nuclear translocation inhibits genome amplification, suggesting that the nuclear localization signal might be in a region critical for RNA replication (13). Functional VPg seems to be complexed with another viral product, protein 6kDa, which may mediate VPg binding to membranes at sites of RNA replication (15, 16).

Several studies have reported on the interaction between the poityvirus VPg protein and the eukaryotic translation initiation factor eIF4E, which is the mRNA 5′ cap-binding protein and in plants occurs in two isoforms (17–21). This interaction seems to be crucial for a productive virus cycle. Natural plant resistance to potyvirus infection has been shown to stem from the inability of VPg to interact with eIF4E, as a result of amino acid
mutations either in VPg (22) or in eIF4E (19, 23). VPg of turnip mosaic virus also interacts with the host poly(A)-binding protein (16). Furthermore, turnip mosaic virus VPg has been shown to interact with a plant protein called potyvirus VPg-interacting protein through the 16 amino acids at the VPg N terminus (24). Reduced expression of potyvirus VPg-interacting protein has been observed to diminish susceptibility to turnip mosaic virus infection, whereas eliminating the interaction with VPg resulted in reduced virus cell-to-cell and systemic movement (24). Finally, it has been shown that VPg of infecting PVY can be phosphorylated by plant kinases (25). Consequently it has been hypothesized that VPg phosphorylation could trigger disassembly of infecting virions and subsequent initiation of potyvirus protein synthesis in infected cells (26).

All of these data show first that VPg is potyvirus virulence factor and second that VPg is a multifunctional protein. We demonstrate here that the structure of VPg of PVY is characteristic of an intrinsically disordered protein, which may help to explain the multiple functions played by this protein in the potyvirus life cycle.

**EXPERIMENTAL PROCEDURES**

**Proteins**—Recombinant PVY VPg (accession number Z29526, potato virus Y strain 0, British isolate) and wheat isoform of eIF4E were expressed and purified as described (21). Both plant eIF4E isoforms are able to interact with PVY VPg; however, the isoform interacts more tightly (21).

**Polyacrylamide Gel Analysis**—The analysis was carried out under denaturing conditions according to Laemmli (27) or under semi-denaturing conditions, that is, with samples suspended without boiling in Laemmli sample buffer devoid of SDS and β-mercaptoethanol.

**Proteolytic Analysis**—VPg (15 μg) was incubated with chymotrypsin (2.5, 12.5, and 60 ng) for 10 min at room temperature in 25 μl of 50 mM
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phosphate buffer, pH 7.0, containing 300 mM NaCl. The reactions were stopped with the addition of Laemmli sample buffer, and the proteins were analyzed using 17% SDS-PAGE. For N-terminal amino acid analysis, proteolytic fragments were transferred onto a Problot (ABI) membrane and sequenced as described (21).

Disorder Prediction—The VPg folding predictions were carried out using two software packages: PONDR VL-XT, XL1_XT and CAN_XT, which predict natural disordered regions (www.pondr.com), and FoldIndex®, which predicts to what extent a given protein sequence is intrinsically unfolded (28). FoldIndex® can be used as a Web Service for remote and automatic data processing by accessing this URL: bioportal.weizmann.ac.il/fldbin/findex?m...xml&sq=SEQUENCE. The analyses have been performed using default values.

Dynamic Light Scattering—Protein samples were dialyzed against prefiltered (0.22-μm Millipore filters) 50 mM Hepes buffer, pH 7.6, containing 200 mM KCl, 2 mM DTT, 0.5 mM EDTA, and 10% glycerol. The samples were placed in a reduced volume cuvette (45 μl; Greiner). The automated measurements were collected with a Zetasizer Nano ZS instrument, using a 2 °C incremental temperature ramp and a 2-min equilibrium delay at each temperature. The data were adjusted using the cumulants method.

Analytical Ultracentrifugation—Sedimentation velocity experiments were performed at 42,000 rpm and 1 °C, using an AN-60 rotor of Beckman XL-I analytical ultracentrifuge and two-channel centerpiece with 1.2-cm path length. Three different VPg preparations were analyzed: VPg purified in the presence (5 mM) or absence of DTT, and VPg was stored for 2 weeks at 4 °C without DTT. Each preparation contained ∼0.15 mg of protein/ml (A_{280 nm} = 0.1) in 20 mM Tris-HCl buffer, pH 7.0, containing 300 mM NaCl. The scans were recorded overnight every 6 min at 280 nm using a 0.003-cm radial spacing.

The data for each preparation were analyzed as a continuous distribution c(s) of sedimentation coefficients, s, with the program Sedfit. The program Sedphat was used to perform a global analysis of the three sets of data (all together) in terms of a globular compact monomer and dimer were calculated with \( R_h = \frac{ff_{min} \times R_{min}}{v} \) using the frictional ratio \( ff_{min} = 1.25 \). \( R_{min} \) is the minimum theoretical value for \( R_h \) corresponding to the anhydrous volume \( vM/N_A = (4/3)\pi R_{min}^3 \).

Far-UV CD—CD spectra of VPg at 25 °C were recorded using a Jobin Yvon CD6 spectropolarimeter. The wavelength range used was 180–260 nm, with a 1-nm interval and an integration time of 4 s in continuous scanning mode. The samples were contained in 0.1-cm optical path length quartz cuvettes. VPg (0.2 mg/ml) was in 50 mM sodium phosphate, pH 7.0, containing 60 mM NaCl with or without 20% TFE. To monitor VPg thermal stability CD spectra were recorded after 30 min of incubation at various temperatures up to 95 °C.

NMR Spectroscopy—Conventional 1H one-dimensional NMR using excitation sculpting for water suppression purposes (31) and one-dimensional HET-SOFAST experiments (32) were performed on a Varian DirectDrive 600 spectrometer equipped with a triple resonance cold-probe. The one-dimensional HET-SOFAST experiments are composed of two data sets recorded with and without a band-selective inversion pulse covering the aliphatic region. The data were acquired at 10 or 30 °C. Data processing was done using the VnmrJ software (Varian, Inc.). The time domain data were multiplied with a 90° phase-shifted sine-bell apodization function and zero-filled to 8192 complex data points prior to Fourier transformation. The reference and saturated intensities were obtained by integrating the spectra from −7.0 to 10.0 ppm.

RESULTS AND DISCUSSION

Structure Prediction and Proteolysis—Full-length PVY polyprotein was analyzed using FoldIndex®. This program furnishes an estimate of the degree of protein disorder. It was developed from the algorithm proposed by Uversky et al. (36) and is based on the mean net charge and hydrophobicity of the polypeptide chain. The results clearly show that most of VPg, which is located between amino acids 336 and 523 of PVY polyprotein, lies in the unfolded region of the polyprotein, in contrast to other PVY proteins: CI, 6K2, and NI-Pro (the C-terminal part of NLa proteins containing the protease domain only) (Fig. 1A). The structure of VPg was also probed experimentally with proteases, and the results were compared with the PONDR disorder prediction. There are 31 consensus cleavage sites for trypsin alone, distributed throughout the VPg amino acid sequence. However, when treated with trypsin or chymotrypsin, VPg was cleaved mainly between residues 11–21 and 41–61 (Fig. 1B, lower panel). Thus, the VPg N-terminal region is predominantly accessible to proteases. We infer that also the C-terminal part of the VPg is cleaved as the N-terminal analysis revealed

FIGURE 1. Disorder prediction and proteolytic analysis. A, FoldIndex® prediction for part of the PVY polyprotein, containing proteins CI, 6K2, VPg, and NI-Pro (NCBI data base). VPg is located between amino acids 336 and 523. Except for small fragments between residues 225 and 275, VPg is the only part of the polyprotein showing disordered nature. B, proteolytic analysis. VPg was cleaved with 2.5, 12.5, and 60 ng of chymotrypsin for 10 min at room temperature in 25 μl of 50 mM phosphate buffer, pH 7.0, containing 300 mM NaCl. The resulting fragments (bands a–d) were identified by their N-terminal sequence as described under “Experimental Procedures.” The lower panel shows experimentally detected chymotrypsin (wedges) and trypsin (arrows, gel not shown) proteolysed sites in VPg amino acid sequence. C, disordered regions (marked with letter D) in VPg amino acid sequence predicted with PONDR program (three different predictors were used: VLXT, XL1_XT, and CAN_XT). The regions that were not predicted as disordered by PONDR are shown as bold letters.
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The protein melting point temperature monitors unfolding or denaturation caused by changes in pH or temperature. The protein melting point temperature reflects the transition temperatures of the denatured polypeptide chains. The change in mean particle size that accompanies protein denaturation was measured using a 12–65°C thermal gradient. In this temperature range the VPg preparation showed no signs of denaturation (Fig. 2B). Parallel measurements done with eIF4E produced a typical denaturation profile with a slow increase in particle size beginning at ~55°C, followed by a rapid increase at 60°C.

**VPg Oligomerization**—VPg contains one cysteine (Cys\textsuperscript{150}) among its 188 amino acid residues. When VPg was prepared under nonreducing conditions and run on semi-denaturing SDS-PAGE, a slowly migrating band of 44 kDa, consistent with the molecular weight calculated for VPg dimer (21) was observed in addition to a major band (22kDa) corresponding to the monomeric protein (Fig. 3A, lane 1). The same preparation run on denaturing PAGE showed only one band migrating at 22 kDa (Fig. 3A, lane 3). The amount of the 44-kDa form increased with time of storage at 4°C but disappeared when VPg was incubated with 5 mM DTT (Fig. 3A, lane 2). When N-ethylmaleimide was added at the beginning of VPg purification, the protein appeared in a monomeric form only (Fig. 3B, lanes 1 and 2). N-Ethylmaleimide forms covalent bonds with sulfhydryl groups of cysteines and prevents S-S bond formation. Similar gel analysis performed on VPg C-terminally truncated at residue 139 showed only one band migrating with the monomer molecular weight under nonreducing conditions (Fig. 3B, lanes 3 and 4). Moreover, no VPg dimers were observed in freshly expressing insect cells (Fig. 3B, lane 6; His-tagged VPg is somewhat retarded in comparison with untagged protein). It seems thus that the VPg dimer arises during VPg purification or manipulation, when atmospheric oxygen induces formation of disulfide bonds. It is worth noting that the RNAs prepared from three different potyviruses were found in an apparently VPg-dependent aggregated form (37). The propensity for oligomerization has been observed previously for VPg proteins of clover yellow vein virus (38) and turnip mosaic virus (14).

The appearance of dimers caused by the formation of disulfide bridges might have changed or impaired VPg functionality. For example, stabilization of *Agrobacterium tumefaciens* VirB7, an outer membrane-associated lipoprotein, is correlated with its ability to form disulfide cross-linked homodimers (39). Similarly, cysteine oxidation prevents p53 dimerization and hence inhibits DNA binding (40). In some redox-responsive proteins, the formation of disulfide bonds modulates protein-protein interactions; upon oxidation *Escherichia coli* chaperone Hsp33 undergoes a large conformational transition to a state that can bind polypeptide substrates and rescue them from aggregation (41). To explore the effect of VPg dimerization on function through a change in interaction with its host partner, eIF4E, we studied the complex formation using monomeric and dimeric VPg in parallel. As shown in Fig. 3C (lanes E), no difference is observed in the interaction of DTT-treated VPg (monomeric form) and stored VPg (dimeric form) with GST-eIF4E immobilized on a GST column. This suggests that VPg utilizes for dimerization regions different from those implicated in its interaction with eIF4E. Note that the N-terminal part of VPg seems to interact with host eIF4E (18, 21), whereas the C-terminal part is involved in dimerization (Fig. 3B, lanes 3 and 4).
Also oligomerization of clover yellow vein virus VPg involves the C-terminal part of the protein (38).

**Analytical Ultracentrifugation**—Sedimentation velocity experiments were performed to evaluate the shape and oligomeric state of VPg under nondenaturating conditions. As mentioned earlier, VPg has a tendency to oligomerize upon prolonged storage. From c(s) analysis (Fig. 4), VPg stored at −20 °C and thawed (VPgM+D sample) has a maximum mean s_{20,w} of 2.5 S. VPg stored for 2 weeks at 4 °C (VPgD sample) and believed to be a dimer has a larger mean s_{20,w} value of 3.0 S, whereas VPg purified in the presence of DTT (monomer, VPgM) has the smallest s_{20,w} mean value of 2.0 S. The three sets of data were also modeled as a mixture of noninteracting monomer and dimer species. In this approach, the molar mass is fixed, whereas the sedimentation coefficient for monomer and dimer, as well as the proportion of both species in different samples, are fitted. The results are compatible with the presence of ~90% monomer in the VPgM sample, ~90% dimer in the VPgD sample, and equal amounts of the monomer and dimer in the VPgM+D sample (Tables 1 and 2). The quality of our data (not shown) does not allow us to distinguish between rapid equilibrium (as suggested by the intermediate position of the VPgM+D peak in Fig. 4) and slow equilibrium (corresponding to an analysis with two species). The results of the monomer/dimer mixture analysis gives the s_{20,w} values 1.7 and 3.0 S for VPg monomer and dimer, respectively. These values are rather small compared with those calculated for globular compact species, namely 2.1 and 3.4 S (Table 2). The results thus imply an elongated structure for both monomeric and dimeric VPg.

**Circular Dichroism**—Intrinsically unstructured proteins possess distinctive far-UV CD spectra with a characteristic deep minima in the vicinity of 200 nm and relatively low ellipticity at 215 and 222 nm resulting from the low content of ordered secondary structure. The CD spectra of monomeric and dimeric VPg (Fig. 5A) are identical and exhibit negative ellipticity near 205 nm, a shoulder at 220 nm, and a weak positive maximum of ~190 nm. These spectral features have been

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**TABLE 1**

| Samples   | Monomer | Dimer |
|-----------|---------|-------|
| VPgM      | 89      | 11    |
| VPgM+D    | 47      | 53    |
| VPgD      | 8       | 92    |

**TABLE 2**

| Molecular mass | Fitted s_{20,w} | Theoretical s_{20,w} with f/f_{max} = 1.25 |
|----------------|-----------------|------------------------------------------|
| Monomer        | 22.2            | 1.7                                      |
| Dimer          | 44.4            | 3.0                                      |
assigned to a group of \(\beta\)-sheet-rich proteins containing short segments of \(\beta\)-strands (so called beta-II proteins) \((42)\). The VPg spectrum was not changed by the presence of 10% TFE but was significantly modified by 20% TFE (Fig. 5A, dots). This spectrum has a positive molar ellipticity, centered at \(\lambda_{200}\) and a negative band at 228 nm, strikingly similar to spectra observed with beta-I rich proteins \((42)\). The effect of temperature was tested (Fig. 5B), and in common with other unfolded proteins \((43)\), no cooperative transition of the molar ellipticity was observed. Our data show that VPg does not have a compact structure, nor does it contain significant secondary elements. In addition, it appears that VPg undergoes a structural reorganization in TFE, which is known to favor local interactions by increasing the propensity for helix formation. Because the dielectric constant of TFE is approximately one-third of that of water, charge-mediated interactions should be stronger in TFE; in particular intramolecular hydrogen bonds should be strengthened by the addition of TFE to an aqueous solution (Ref. 44 and the references therein).

**NMR Spectroscopy**—The degree of dispersion of the proton signals observed by one-dimensional NMR reflects the extent of protein folding. Recently, a new NMR experimental procedure, called HET-SOFAST, has been proposed by Schanda et al. \((32)\), which allows the extent of protein compactness to be quantified using the ratio, \(\lambda_{\text{NOE}}\) (measure of average proton density), between a reference and a saturated spectrum. Although for compact, well folded proteins \(\lambda_{\text{NOE}}\) falls between 0.1 and 0.4, it increases up to 0.8 for totally unstructured proteins. HET-SOFAST experiments were applied to monomeric and dimeric samples of VPg, at 10 and 30 °C. The \(^1\)H NMR spectra recorded under these different conditions were quite similar and showed a relatively poor dispersion of the amide protons in the 6–9 ppm region (Fig. 6A), characteristic of a rather unfolded protein. However, several aliphatic peaks were observed at \(\approx 0\) ppm, located away from the random coil frequency at \(\approx 0.7\) ppm of the main peak. These peaks most likely arise from regularly folded regions. The \(\lambda_{\text{NOE}}\) parameters of a protein in the monomeric state were \(\approx 0.5\), independently of the conditions. These values of \(\lambda_{\text{NOE}}\) are characteristic of partially unfolded or degraded proteins \((32)\). Because the VPg samples were found not to be degraded after the NMR experiment (data not shown), these data confirm that VPg is in an unfolded state.

Both the NMR (Fig. 6B) and CD spectra indicate that there is no significant change in VPg structure in the presence of 10% TFE. In contrast, CD spectra taken in the presence of 20% TFE point to a gross structural change (Fig. 5A). Therefore, we attempted to carry out the NMR experiment at 20% TFE. However, the analysis was precluded because these conditions caused VPg aggregation. It should be noted that NMR measurements require significantly higher protein concentrations (3 \(\mu\)g/\(\mu\)l) than CD measurements (0.3 \(\mu\)g/\(\mu\)l). VPg aggregates obtained in 20% TFE dissolved upon incubation for 3 min at 100 °C and reformed during cooling. This treatment could be...
repeated several times. It seems that local interactions in the presence of TFE result in the acquisition of a secondary structure and lead to VPg aggregation, whereas the weakening of local interactions caused by the increase in temperature results in resolubilization.

Potyvirus VPgs do not have homologs, and their atomic structure is unknown. Our attempts to obtain PVY VPg crystals have been unsuccessful, which we attribute at least in part to the intrinsically disordered nature of the protein revealed by our data. Taken together, the physicochemical data indicate that VPg has an elongated tertiary structure dominated by many disordered regions. Additionally, VPg dimerization, through the interaction of active cysteines, emphasizes its interactive propensity. It is conceivable that the plasticity of VPg resulting from the largely unstructured state allows it to easily interact with various partner proteins. So far, regions lacking ordered three-dimensional structures have been mainly found in proteins involved in regulatory and signaling events (2, 45–47). It is thus possible that VPg is also a regulatory protein involved in more processes than is currently believed.

The experimental data on the structure of two other, albeit quite small, VPgs suggest that the unordered state is perhaps a common feature of this class of proteins. NMR studies on cowpea mosaic virus VPg imply the absence of an ordered structure (48). Similarly, the CD and fluorescence spectra obtained from Sesbania mosaic virus VPg indicate a lack of tertiary structure (49). However, because VPg of cowpea mosaic virus and Sesbania mosaic virus consists of 28 and 77 amino acid residues, respectively, it is not clear that general conclusions can be drawn from such a small sample of proteins with such disparate lengths. Therefore, we used the Foldindex© program to analyze the structure of several viral terminal proteins from both DNA and RNA virus genomes.
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and RNA viruses were analyzed (Tables 1 and 2). Initially, two measles virus proteins were analyzed. These are not genome-linked proteins but have been shown experimentally to contain large unstructured regions (50). The program predicted that they are between 30 and 40% unfolded (Table 3). In particular, the C-terminal domain of measles virus nucleoprotein, N\textsubscript{TAIL} (amino acids 401–525), was shown to belong to the class of intrinsically disordered proteins (51) and the Foldindex\textsuperscript{©} assigns two disordered regions to this domain of 124 amino acids, containing a combined total of 112 amino acids. The degree of unfolding of three plant virus VPg proteins of similar length is predicted to be very different. PVY VPg might contain as much as 75% of putative disordered regions, whereas another potyvirus (tobacco etch virus) VPg is predicted to contain only approximately half as much. An interesting case is presented by the terminal protein of bacteriophage phi29, which is estimated to be nearly completely unfolded. Finally, wheat eIF(iso)4E, a high affinity partner of PVY VPg (21), is predicted to have a low but significant percentage of disorder. Of note, the crystallogenesis of eIF4E was rather unsuccessful until it was co-crystallized with m’GTP ligand (52). Thus, three proteins with a rather high Foldindex\textsuperscript{©} score, the phosphoprotein and nucleocapsid-binding domain of Sendai virus phosphoprotein P, is disordered (54). In addition, a high degree of disorder has been predicted for viral proteins such as Ebola virus nucleoprotein and adenovirus minor capsid protein VI (55), but no experimental data exist yet. All of these examples, including VPg, are multifunctional proteins, interacting with a variety of viral as well as host partners. A large degree of flexibility would combine high specificity with low affinity to ensure faster association and dissociation rates, enable binding of numerous structurally distinct targets, and provide the ability to overcome steric restrictions, thus leading to larger surfaces of interaction between partners (33, 35, 56–59). It is relevant in this respect that adaptation and survival of viruses that contain only restricted genetic information depend on the multifunctionality of their components. Viruses have evolved to maximize their genomic information by producing multifunctional proteins.

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REFERENCES
1. Romero, P., Obradowic, Z., Kissinger, C. R., Villafranca, J. E., Garner, E., Guilliot, S., and Dunker, A. K. (1998) Pac. Symp. Bioinform. 437–448
2. Tompa, P. (2002) Trends Biochem. Sci. 27, 527–533
3. Herbert, T. P., Brierley, I., and Brown, T. D. (1997) J. Gen. Virol. 78, 1033–1040
4. Murphy, J. F., Klein, P. G., Hunt, A. G., and Shaw, J. G. (1996) Virology 220, 535–538
5. Mitra, T., Bosso, S. V., and Green, K. Y. (2004) J. Virol. 78, 4931–4935
6. Lellis, A. D., Kasschau, K. D., Whitham, S. A., and Carrington, J. C. (2002) Curr. Biol. 12, 1046–1051
7. Schaad, M. C., Lellis, A. D., and Carrington, J. C. (1997) J. Virol. 11, 8624–8631
8. Fellers, J., Jan, J., Hong, Y., Collins, G. B., and Hunt, A. G. (1998) J. Gen. Virol. 79, 2043–2049
9. Hong, Y., Levay, K., Murphy, J. F., Klein, P. G., Shaw, J. G., and Hunt, A. G. (1995) Virology 214, 159–166
10. Puustinen, P., and Makinen, K. (2004) J. Virol. 78, 38103–38110
11. Restrepo, M. A., Freed, D. D., and Carrington, J. C. (1990) Plant Cell 2, 987–998
12. Carrington, J. C., Freed, D. D., and Leinicke, A. J. (1991) Plant Cell 3, 953–962
13. Schaad, M. C., Haldeman-Cahill, R., Cronin, S., and Carrington, J. C. (1996) J. Virol. 70, 7039–7048
14. Beauchemin, C., Boutet, N., and Laliberte, J. F. (2007) J. Virol. 81, 775–782
15. Restrepo-Hartwig, M. A., and Carrington, J. C. (1994) J. Virol. 68, 2388–2397
16. Léonard, S., Viel, C., Beauchemin, C., Daigeneault, N., Fortin, M. G., and Laliberté, J. F. (2004) J. Gen. Virol. 85, 1055–1063
17. Wittmann, S., Chatel, H., Fortin, M. G., and Laliberte, J. F. (1997) Virology 234, 84–92
18. Léonard, S., Plante, D., Wittmann, S., Daigeneault, N., Fortin, M. G., and Laliberté, J. F. (2000) J. Virol. 74, 7730–7737
19. Duprat, A., Caranta, C., Revers, F., Menand, B., Browning, K. S., and Robaglia, C. (2002) Plant J. 3, 927–934
20. Kang, B. C., Yeom, I., Frantz, J. D., Murphy, J. F., and Jahn, M. M. (2005) Plant J. 42, 392–405
21. Grzela, R., Strokovska, L., Andrieu, J. P., Dublet, B., Zagorski, W., and Chroboczek, J. (2006) Biochimie (Paris) 88, 887–896
22. Borgstrom, B., and Johansen, I. E. (2001) Mol. Plant Microbe Interact. 14, 707–714
23. Ruffel, S., Dussault, M. H., Palloix, A., Moury, B., Bendahmane, A., Robaglia, C., and Caranta, C. (2002) Plant J. 32, 1067–1075
24. Dunoyer, P., Thomas, C., Harrison, S., Revers, F., and Maule, A. (2004) J. Virol. 78, 2301–2309
25. Ivanov, K. I., Puustinen, P., Merits, A., Saarma, M., and Makinen, K. (2001) J. Biol. Chem. 276, 13530–13540
26. Puustinen, P., Rajamaki, M. L., Ivanov, K. I., Valkonen, J. P., and Makinen, K. (2002) J. Virol. 76, 12703–12711
27. Laemmli, U. K. (1970) Nature 227, 680–685
28. Prilusky, J., Zeev-Ben-Mordehai, T., Rydberg, E., Felder, C., Silman, I., and Sussman, J. L. (2005) Bioinformatics 21, 3435–3438
29. Schuck, P. (2000) Biophys J. 78, 1606–1619
30. Ebel, C. (2007) Protein Structures: Methods in Protein Structure and Stability Analysis, Nova Science Publishers, Inc., Hauppauge, NY
31. Schuck, P. (2000) Biophys J. 78, 1606–1619
32. Hwang, T. L., and Shaka, A. J. (1995) J. Magn. Reson. A 125, 729–737
33. Schraud, P., Forge, V., and Brutscher, B. (2006) Magn. Reson. Chem. 44, 5177–5184
34. Uversky, V. N. (2002) Eur. J. Biochem. 269, 2–12
35. Deleted in proof
36. Uversky, V. N., Gillespie, J. R., and Fink, A. L. (2000) Proteins 41, 415–427
37. Luciano, C. S., Murphy, J. F., Rhoads, R. E., and Shaw, J. G. (1991) J. Gen. Virol. 72, 205–208
38. Yambo, M. L., Masuta, C., Nakahara, K., and Uyeda I. (2003) J. Gen. Virol. 84, 2861–2869
39. Spudich, G. M., Fernandez, D., Zhou, X. R., and Christie, P. J. (1996) Proc. J.].
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