

# Changes in the Structure of Potato Virus A Virions after Limited *in situ* Proteolysis According to Tritium Labeling Data and Computer Simulation

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**Abstract**—Coat proteins (CP) of the potato virus A virions (PVA) contain partially disordered N-terminal domains, which are necessary for performing vital functions of the virus. Comparative analysis of the structures of coat proteins (CPs) in the intact PVA virions and in the virus particles lacking N-terminal 32 amino acids (PVA $\Delta$ 32) was carried out in this work based on the tritium planigraphy data. Using atomic-resolution structure of the potato virus Y potyvirus (PVY) protein, which is a homolog of the CP PVA, the available CP surfaces in the PVY virion were calculated and the areas of intersubunit/interhelix contacts were determined. For this purpose, the approach of Lee and Richards [Lee, B., and Richards, F. M. (1971) *J. Mol. Biol.*, **55**, 379-400] was used. Comparison of incorporation profiles of the tritium label in the intact and trypsin-degraded PVA $\Delta$ 32 revealed position of the  $\Delta$ N-peptide shielding the surface domain (a.a. 66-73, 141-146) and the interhelix zone (a.a. 161-175) of the PVA CP. Presence of the channels/cavities was found in the virion, which turned out to be partially permeable to tritium atoms. Upon removal of the  $\Delta$ N-peptide, decrease in the label incorporation within the virion (a.a. 184-200) was also observed, indicating possible structural transition leading to the virion compactization. Based on the obtained data, we can conclude that part of the surface  $\Delta$ N-peptide is inserted between the coils of the virion helix thus increasing the helix pitch and providing greater flexibility of the virion, which is important for intercellular transport of the viruses in the plants.

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**Keywords:** potyviruses, coat protein, potato virus A, N-terminal disordered domains, structural modeling, tritium planigraphy

## INTRODUCTION

Potyviruses comprise an important group of plant viruses that cause significant economic losses in agriculture. Hence, investigation of their structure and functions is in the spotlight. Flexible filamentous virions of potyviruses have helical symmetry with average particle length of 700 nm and diameter 11-14 nm. It was shown with the help of a number of methods of structural analysis and modeling that the coat proteins (CP) of potyvi-

ruses are partly disordered, which also complicates their examination [1, 2]. There is a variety of methods for determination of the structure of viral particles including physicochemical techniques and methods of electron microscopy (EM), especially cryogenic EM (cryo-EM). Despite the complexity of investigation of flexible particles, combination of investigation of the properties of mutant viruses, as well as progress in the area of computational techniques and image processing [1, 3-5] resulted in better understanding of the structure of potyviruses and of the role of the fragments of their CP in the virus life cycle.

The first model of the CP of the typical representative of potyviruses, potato virus A (PVA) was suggested in 2001 [3]. Viral particles were bombarded with the

*Abbreviations:* CP, coat proteins; EM, electron microscopy; PVA, potato virus A; PVA $\Delta$ 32, PVA virion lacking 32 amino acids at N-terminus; PVY, potato virus Y.

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thermally activated tritium atoms and intramolecular distribution of the label in the CP amino acids was determined in order to evaluate their steric accessibility *in situ*. The model of spatial arrangement of the PVA CP polypeptide chain inside the virion was suggested based on the experimental data obtained during bombardment with tritium together with predictions of the secondary structure elements and principles of folding of helices and structural elements in the proteins. The model predicts existence of three domains of the tertiary structure: (i) accessible from outside N-terminal domain consisting of 8-aa unstructured N-terminus and two  $\beta$ -strands, (ii) C-terminal domain including two helices and three  $\beta$ -strands that form two-layer structure termed abCd block, and (iii) central domain containing a four-helix bundle similar the one found in the CP of the tobacco mosaic virus. For many years it was the only model used for describing three-dimensional structure of the CP of potyviruses.

Use of cryogenic electron microscopy (cryo-EM) is an important milestone in the development of our notion on the structure of potyviruses. Three-dimensional structure of the virions of several potyviruses was determined with the help of this technology – watermelon mosaic virus (WMV) [6], potato virus Y (PVY) [4], turnip mosaic virus (TuMV) [7]. These high-resolution structures confirmed existence of conserved three-domain architecture common for non-related potyviruses, which is different from the hypothetical structure of the PVA [3]. The obtained atomic structures of WMV, PVY, and TuMV have identical left-handed helical arrangement of CP subunits in the virion, which consisted of a central core with seven  $\alpha$ -helices and one or two  $\beta$ -hairpins, as well as N- and C-terminal domains without pronounced secondary structure. The structures of flexible disordered N-terminal peptides exposed to the solvent were not resolved likely due to its disordered nature and high mobility, hence, first 59, 43, or 65 amino acid residues are absent in the atomic structures of WMV, PVY, and TuMV, respectively. In all three potyviruses the surface-localized electropositive N-terminal domains interacted with electronegative grooves of the neighboring CP molecules establishing side contacts and mediating polymerization of the particles similar to potexviruses [8, 9]. It is likely that flexibility and disorder of the N-terminal domains in combination with contacts with the neighboring subunits enable additional flexibility of the virions [4, 6] required, e.g., for facilitation of intercellular transfer [10].

It is important to mention that in the course of isolation and storage of potyvirus preparations very often natural *in situ* proteolysis with trypsin-like proteases from the cellular juice occurs resulting in the deletion of 30-50 N-terminal residues ( $\Delta$ N-peptides) [11, 12]. In the process such viral particles remain infective. Biological significance of this phenomenon is presumably

associated with the necessity of the release of infective particles from the aphids stilettoes for further transfer to plants [13, 14].

The main goal of this study was determination of exact location of  $\Delta$ N-peptides on the surface of PVA virions and elucidation of the effect of their deletion on the possible changes of the virion structure. Due to flexibility and partial disorder of the CP in PVA the method of tritium planigraphy was found to be suitable for this investigation [15]. This approach is based on non-selective replacement of hydrogen in hydrocarbon fragments of the molecules with its radioactive isotope, tritium. Tritium is used as a nanoprobe with an effective radius of 0.09 nm for determination of amino acid residues localized on the virion surface, as well as in the cavities accessible to tritium [16-18]. The method is based on chemical reactions with participation of hot tritium atoms; reaction conditions have been selected that result in incorporation of the probe as a result of a single collision, which characterize steric accessibility of C-H bonds in the virion composition. The data on steric accessibility of the system components obtained with the help of this method characterize the structure of the whole object. The method of tritium planigraphy [15] has been successfully used for examination of spatial organization of proteins and their complexes, as well as of highly organized biological objects such as ribosome [19], plant and animal viruses [3, 20-22]. Bombardment of the tested object with a beam of tritium atoms followed by the analysis of intramolecular distribution of the probe allows determination of the accessible fragments – amino acid residues comprising its surface, which is closely associated with the tertiary structure. In this study the structure of trypsinized virions of the potato virus A with deleted 32 aa at the N-terminus (PVA $\Delta$ 32) was characterized in detail based on the analysis of the results of bombardment of the surface with tritium atoms and precise localization of the  $\Delta$ N-peptides on the surface of PVA virions was determined.

## MATERIALS AND METHODS

**Preparation of samples of intact and partially degraded PVA virions.** The isolate B11 of PVA was purified as described previously [3]. To prepare samples of degraded virions (PVA $\Delta$ 32) preparation of an intact virus were treated with trypsin at the enzyme/substrate ratio 1 : 500 (w/w) followed by 15-min incubation at room temperature, the reaction was stopped by adding phenylmethylsulfonyl fluoride to concentration 1 mM. Purification of the preparation was carried out by precipitation of virions in ultracentrifuge (L-2, Beckman, USA) at 105,000g (100 min, 5°C). Concentration of PVA preparations was determined using absorption coefficient  $E^{0.1\%}_{260\text{ nm}} = 2.3$ . Absorption spectra in UV range

240–340 nm were recorded with a Hitachi UV-2600 spectrophotometer (Hitachi, Japan) in cuvettes with optical path length 0.1–1.0 cm. True absorption spectra (E) of the light-scattering suspensions were calculated using extrapolation method [23]. In all experiments only PVA preparations with normal ratio  $E_{260}/E_{280}$  (~1.25) were used, which indicated absence of defective viral particles. Purity of samples was controlled with the help of sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) according to Laemmli [24] using a 15% polyacrylamide gel (PAAG) in a Mini-PROTEAN 3 Cell (Bio-Rad, USA). The results of transmission electron microscopy (TEM) indicated absence of any defective virions in the initial and trypsin-treated preparations [5].

**Incorporation of tritium.** An aliquot (1 ml) of a virus suspension with concentration of 1–2 mg/ml in 0.05 M borate buffer (pH 8.0) was sprayed on the walls of a reaction vessel cooled with liquid nitrogen, air was removed from the vessel under vacuum, and the vessel was filled with molecular tritium to pressure of 0.5 Pa. Heating of a spiral-shaped tungsten wire located along the central axis of the vessel to 2000 K resulted in dissociation of molecular tritium into atoms, and the formed tritium atoms bombarded suspension of virions cooled with liquid nitrogen. The wire was light up for 15 s followed by removal of residual tritium. The procedure of tritium introduction-removal was repeated two more times. Following introduction of the label, the virus suspension was defrosted and concentrated with centrifugation (105,000g, 5°C, 100 min). To remove the exchangeable label, virus preparations were subjected to 1–2 cycles of virion precipitation at 105,000g. Proteins from the purified virus preparation were isolated using a standard method of extraction with lithium chloride [25]. Purity of preparation was evaluated with PAGE [24].

**Enzymatic hydrolysis of proteins.** The obtained protein preparations were subjected to tryptic hydrolysis. Trypsin solution (TPCK-trypsin, Sigma, USA) was added to protein solutions at a ratio enzyme/substrate 1 : 50 (w/w), and hydrolysis was carried out at 37°C for 4 h; reaction was stopped by adding phenylmethylsulfonyl fluoride to concentration 1 mM.

**Analytical techniques.** Mixture of peptides obtained as a result of hydrolysis was separated with the help of Beckman high-performance liquid chromatography system on a reversed-phase column Ultrasphere ODS, 5  $\mu$ m, 250  $\times$  4.6 mm, (Beckman, USA). All separations were performed using linear gradients of acetonitrile in water containing 0.1% trifluoroacetic acid (0 to 60% in 60 min and 60 to 80% in 10 min) with flow rate 1 ml/min. Detection was carried out based on absorption at wavelengths 215 and 280 nm. Fractions corresponding to individual peptides were collected for further analysis. Peptides were subjected to acid hydrolysis as described previously [26].

Acidic hydrolysates were analyzed with an Amino Acid Analyzer L8800 (Hitachi) equipped with a cation-exchange column using ninhydrin derivatization with modifications [27]. Simultaneous measurements of radioactivity were carried out with a Radiomatic 150TR Flow Scintillation Analyzer (Packard Co., USA). Obtained data (optical density of an eluate at 570 and 440 nm and radioactivity) were processed using Multi-Chrom for Windows software (Ampersand Ltd., Russia). Next molar radioactivity of each amino acid residue was calculated [28].

**Calculation of accessible surface of the virion** was carried out using the GROMACS 5.1 software package [29]. Only part of the surface occupied with hydrogen atoms linked to carbon atoms (CH-surface) was considered; part of the surface occupied by other atoms was ignored. Radius of the probe was 0.09 nm. To determine regions of intermolecular contacts, surface of an individual subunit was calculated using the same approach followed by evaluation of the fraction of the surface shielded as a result of assembly of the viral particle. RasMol program (<http://www.rasmol.org/>) was used for visualization of protein structures.

## RESULTS

**Investigation of PVA and PVA $\Delta$ 32 virus particles with tritium planigraphy.** In our earlier studies we observed that storage of intact preparations of PVA results in cleavage of CP molecules *in situ* by proteases from the cellular juice at positions 32 and 42 [11]. Limited trypsinolysis of the intact virus preparations (at enzyme/substrate ratio 1 : 500, 15 min) also resulted in the cleavage of 32 N-terminal residues of the virion CP, which was confirmed with the results of mass spectrometry and SDS-PAGE [11]; according to the TEM data the virions maintained their integrity [5]. In this study we conducted comparative structural analysis of two types of preparations: intact PVA virions and partially degraded with trypsin virions PVA $\Delta$ 32 (see Materials and Methods). Distribution of the tritium probe along the polypeptide chain after labeling of intact PVA and PVA $\Delta$ 32 with tritium is shown in Fig. 1. The results of our investigation of the intact PVA with the help of tritium planigraphy have been published previously [3]. To determine distribution of the label along the polypeptide chain of the intact PVA CP and its truncated variant all peptides obtained by trypsin-mediated hydrolysis except three short peptides T6, T7, T21, and part of T11 were isolated and analyzed (Fig. 1).

The levels of specific radioactivity of the residues were corrected considering corresponding coefficients [30] to make them proportional to accessible surfaces. Construction of a histogram in percent of total specific radioactivity ( $A_{sp}$ , %) or values specific radioactivity normalized

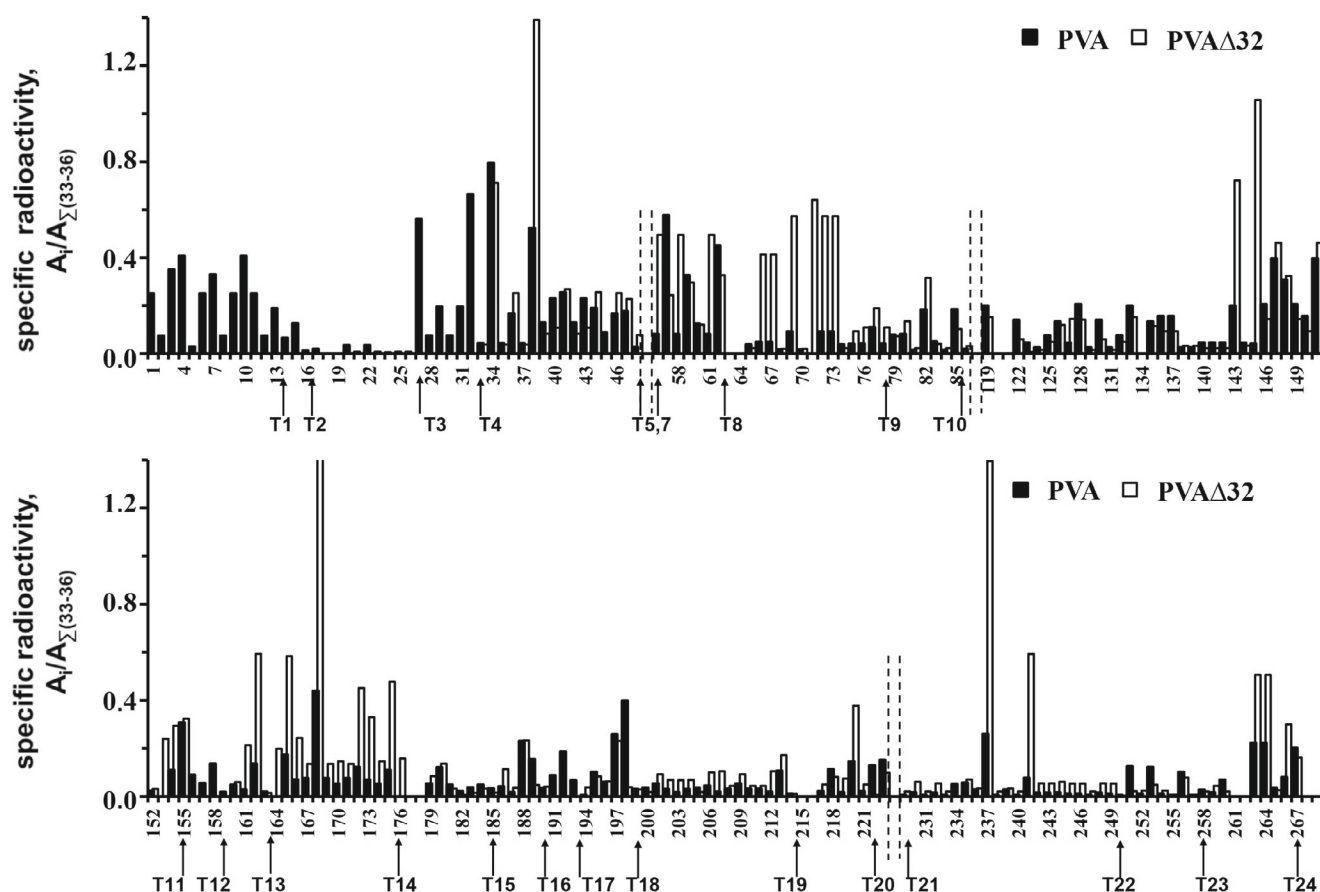


Fig. 1. Distribution of tritium label over amino acid residues of proteins in PVA and PVA $\Delta$ 32 virions with consideration reactivity of tritium atoms in reactions with different amino acids.  $A_i/A_{\Sigma(33-36)}$  – specific radioactivity of the residue ( $A_i$ ) normalized to the sum of specific radioactivities of the surface residues 33-36. Numbering of amino acid residues is shown in abscissa axis. Sites of cleavage by trypsin marked with arrows. Data for cysteine and tryptophan are not shown as they are destroyed with acid hydrolysis. Positions of missing peptides are shown with dashed lines. Graph is presented in two parts (upper and lower) for better visualization.

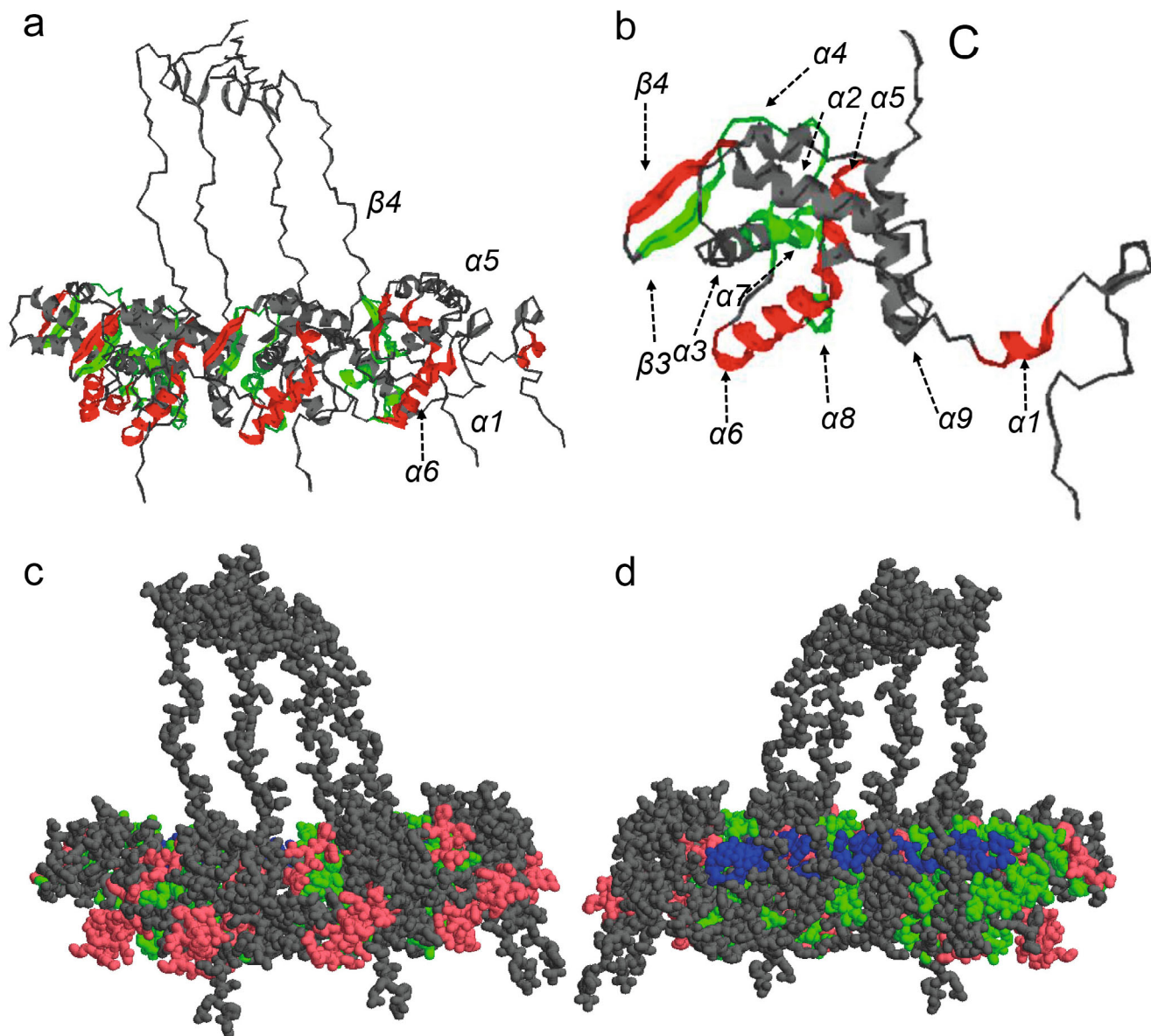
to one or several (33–36 aa) surface residues in the vicinity of the proteolysis site gave similar results. As can be seen in Fig. 1, distribution of the tritium label in the intact PVA virions and in the PVA $\Delta$ 32 is significantly different.

In the PVA $\Delta$ 32 CP the levels of specific radioactivity of the residues 66–73, 141–146, 161–175, 233–247 increased in comparison with the intact PVA, and of the residues 122–138, 184–191, 195–200 – decreased (Fig. 1). In order to understand in what regions of the CP tertiary structure these changes occur, we used the available 3D structure of PVY CP, which is a close homolog of PVA CP. Primary structures of CPs from PVA and PVY exhibit significant similarity: 64% of identical and 91% of similar residues (of 237 CP residues) [5]. Sequence of the N-terminal domain in the PVA CP is longer by 2 residues than in the PVY CP. Three-dimensional structure of the PVY CP (PDB ID: 6HXX, residues 44–267) obtained using cryo-EM with resolution 0.34 nm included three domains: N-terminal domain (I), residues 44–77; central domain (II), residues 78–225; and C-terminal domain (III), residues 226–267 (Fig. 2). The domain I protrudes from the central domain, and, according to

the structure derived from the cryo-EM data, surrounds the domain II of two neighboring CP. Atomic coordinates of the residues 1–43 were not determined presumably due to partial disorder and mobility of this region. High level of similarity between the primary structures of CP from PVA and PVY allowed using the PVY CP structure (PDB: 6HXX) for modeling the structure of coat protein in PVA.

We calculated incorporation of the tritium label (in % of total radioactivity) into the elements of secondary structure of the protein [4] for the virions PVA and PVA $\Delta$ 32; the results are presented in the table.

Total calculated radioactivity of the element ( $A$ ) in the virus particles PVA $\Delta$ 32 and PVA, as well as relative change of radioactivity  $A_{PVA\Delta32}/A_{PVA}$  are presented in the table. It can be seen that removal of the N-terminal CP fragment in the PVA $\Delta$ 32 results in the increase of accessibility of the helices  $\alpha 1$  (68–74 aa),  $\alpha 5$  (157–163 aa),  $\alpha 6$  (165–180 aa), and of the  $\beta 4$ -strand (141–146 aa). At the same time, tritium accessibility to the fragment  $\alpha 3$ -14- $\beta 3$  (119–138 aa) and of the helices  $\alpha 7$  (184–191 aa) and  $\alpha 8$  (195–200 aa) decreases. Relative change of the



**Fig. 2.** Relative change of radioactivity in the secondary structure elements of the proteins in PVA occurring due to removal of the  $\Delta$ N-peptides. Regions with ratio  $A_{PVA\Delta 32}/A_{PVA} > 1.8$  are shown in red,  $< 0.5$  – in green. a) Fragment consisting of four subunits; b) fragment consisting of one subunit (helices are shown as ribbons); C, C-end of CP; c, d) four subunits are shown (skeleton of the structure) from outside and from inside of the virion, respectively; RNA is marked with blue (d).

virion radioactivity in the elements of secondary structure of the protein due to removal of the  $\Delta$ N-peptide is demonstrated using the 3D structure model of PVY (PDB: 6HXX) with two colors for better visualization (Fig. 2). Structural fragments with increased accessibility to tritium are shown with red color; fragments with decreased accessibility to tritium with green color.

The fact that increases of the label incorporation due to deletion of  $\Delta$ N-peptides is observed on the virion surface (Fig. 2c) attracted out attention. Up to 34% and 25% of total radioactivity was incorporated into the surface domain I (33-79 aa) of the PVA $\Delta$ 32 and PVA, respectively. This indicates shielding of the domain I by

the  $\Delta$ N-peptide. The  $\Delta$ N-peptide in the intact PVA exhibited the highest level of labelling in the CP, contained 22% of the total amount of the label, and comprised 12% of the CP length. The tritium label only insignificantly was incorporated into the inner layers of the virion (Fig. 2d), which implied penetration of tritium atoms through inter-subunit contacts and/or any other channels in the virion. Removal of the  $\Delta$ N-peptide resulted in the decrease of the level of labeling of the inner part of the virion (Fig. 2d), which suggested structural rearrangements resulting in compaction of the virion.

**Calculation of accessible surface of CP in the virion and in free state.** To determine inter-subunit and interhe-

Distribution of tritium label over the elements of CP secondary structure in the virions PVA and PVA $\Delta$ 32, and accessible area of the CH-surface calculated with the help of GROMACS 5.1 program using PVY model (PDB: 6HXX)

Element of secondary structure	Nos. of residues	Number of residues	Total radioactivity of the element, A <sup>a</sup> , %		Relative change, S <sup>b</sup>	Accessible area of CH-surface, S <sup>c</sup> , nm <sup>2</sup>		Fraction of inaccessible area of CH-surface, S <sup>b</sup> (S <sub>CP PVY</sub> - S <sub>PVY</sub> )/S <sub>CP PVY</sub>
			PVA $\Delta$ 32	PVA		A <sub>PVA<math>\Delta</math>32</sub> /A <sub>PVA</sub>	PVY CP	
<i>I1</i>	33-59	33	18.7	24,8	0.8	9.2	5.2	<b><u>0.43</u></b>
<i><math>\beta</math>1,2</i>	60-65	6	3.3	4.1	0,8	2.7	1.9	0.28
<i><math>\alpha</math>1</i>	68-74	7	6.1	2.0	<b><u>3.0</u></b>	3.3	2.2	0.31
<i>I2</i>	75-87	13	4.2	5.1	0.8	4.6	2.5	<b><u>0.45</u></b>
<i><math>\alpha</math>3*</i>	119-124	5	0.8	2.4	0.3	0.8	0.5	0.42
<i>I4</i>	125-132	8	2.0	4.3	0.5	2.0	1.1	0.41
<i><math>\beta</math>3</i>	133-138	6	1.7	3.9	0.4	2.0	1.0	<b><u>0.51</u></b>
<i><math>\beta</math>4</i>	141-146	6	6.8	3.4	<b><u>2.0</u></b>	2.0	1.4	0.30
<i><math>\alpha</math>4</i>	147-154	8	6.2	9.4	0.7	1.9	1.5	0.23
<i><math>\alpha</math>5</i>	157-163	7	3.0	1.4	<b><u>2.2</u></b>	0.9	0.6	0.34
<i><math>\alpha</math>6</i>	165-180	16	16.8	8.9	<b><u>1.9</u></b>	4.9	2.3	<b><u>0.52</u></b>
<i><math>\alpha</math>7</i>	184-191	8	1.8	3.3	0.5	2.3	1.1	<b><u>0.50</u></b>
<i><math>\alpha</math>8</i>	195-200	6	1.6	5.3	0.3	1.0	0.8	0.20
<i>I5</i>	201-211	11	2.6	2.3	1.1	1.7	1.3	0.27
<i><math>\alpha</math>9</i>	212-228	17	3.5	4.4	0.8	7.5	3.3	<b><u>0.56</u></b>
<i>C-</i>	229-267	39	15.7	11.5	1.4	20.4	9.6	<b><u>0.53</u></b>

Notes. <sup>a</sup> Total radioactivity of the element was evaluated by summing up radioactivity of all amino acid residues in its composition.

<sup>b</sup> Values A<sub>PVA $\Delta$ 32</sub>/A<sub>PVA</sub> > 1.8 and (S<sub>CP PVY</sub> - S<sub>PVY</sub>)/S<sub>CP PVY</sub> > 0.43 are underlined and shown in bold.

<sup>c</sup> Total accessible area of CH-surface of the element (see below) was calculated with the help of GROMACS 5.1 program by summing up accessible surface of all amino acids in the element composition.

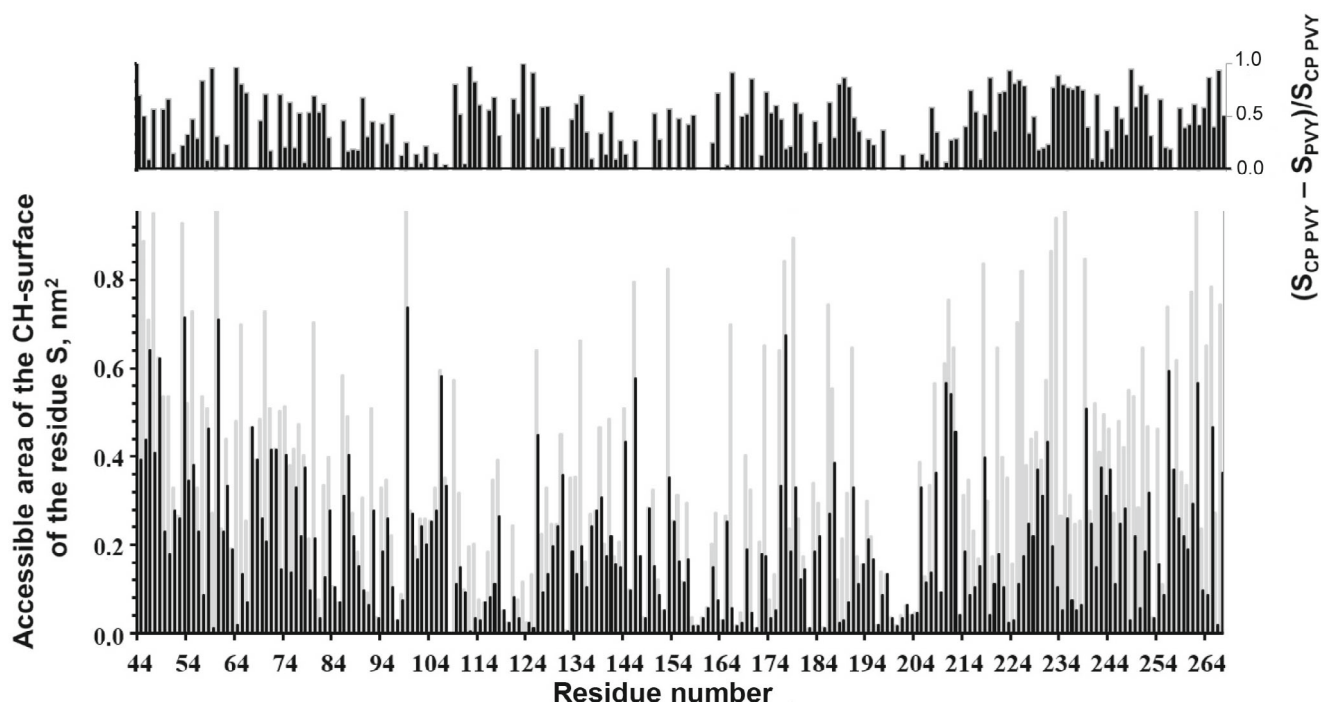
\* Data for the helix  $\alpha$ 2 and part of helix  $\alpha$ 3 are absent.

lix elements of CP and, in addition, to evaluate the obtained experimental data, calculations of the accessible surface of amino acid residues of CP in the virion and in the free CP were carried out for the PVY model based on the data of cryo-EM structure (PDB: 6HXX). Calculations were performed with the help of GROMACS 5.1 program [29] that uses algorithms based on the approach suggested by Lee and Richards [31]. Molecular surface is defined as its part accessible to solvent molecules represented by spheres. Atomic sizes are determined by their Van der Waals radiuses. One free CP protein subunit and a virion fragment consisting of 70 CP (8 turns) were used for calculations. To compare experimental data on triti-

um labeling of the PVA virion with the results of calculations a sphere with 0.09-nm radius was used to model tritium atom as suggested previously [17]. Accessible surface was determined only for the carbon atoms linked to at least one hydrogen atom (CH-surface).

The results of calculations of the accessible CH-surface are presented in Fig. 3 for CP in a free state, S<sub>CP PVY</sub>, and in the composition of virion, S<sub>PVY</sub>.

Total accessible area of the CH-surface in the free protein is 81.6 nm<sup>2</sup>, and in the protein in virion - 44.9 nm<sup>2</sup>. Hence, in the virion 45% (36.7 nm<sup>2</sup>) of the CP surface form contacts between the subunits (not considering 43 N-terminal residues).



**Fig. 3.** Calculations of accessible CH-surface for CP in a free state,  $S_{CP\ PVY}$  (gray bars), and in the composition of virion,  $S_{PVY}$  (black bars), in  $\text{nm}^2$ . Inset: Fraction of the surface of residues of hydrocarbon fragments,  $(S_{CP\ PVY} - S_{PVY})/S_{CP\ PVY}$ , shielded in the virion composition (right axis) relative to the regions of inter-subunit and interhelix contacts of CP in the virion composition.

Relative change of the accessible CH-surface area in the elements of the free protein and in the protein in virion was demonstrated using 3D-model of the PVY CP (Fig. 4, table). Fragments of the surface domain I and central domain II with increased area of CH-surface (Fig. 4, a-c) are shown in red color. Increase of accessibility inside the virion in the C-domain is shown in yellow (Fig. 4d). The calculated total areas of CH-surface in the secondary structure elements,  $S$ , for the free protein and protein in virion are presented in the table, as well as the fraction of inaccessible CH-surface of the virion  $(S_{CP\ PVY} - S_{PVY})/S_{CP\ PVY}$ . It can be seen in the table that the accessible CH-surface of the loops  $I1$  and  $I2$ , belonging to the surface domain I, of the  $\beta3$ -strand, and of the helices  $\alpha6$  and  $\alpha7$  is almost 2-fold larger in the free protein in comparison with the protein in virion, which confirms their location at the site of CP contact with the next turn of the helix.

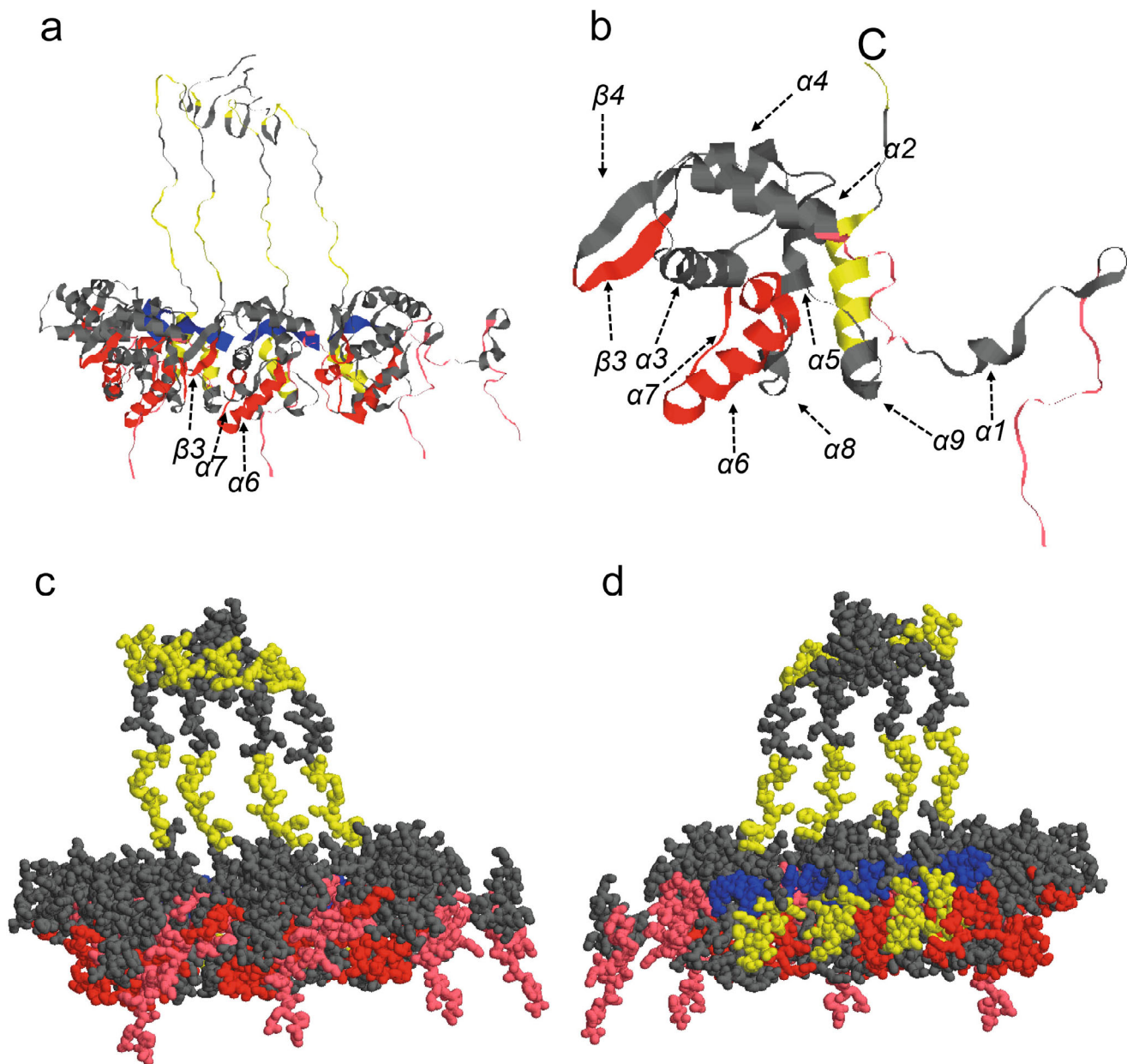
Significant accessible CH-surface of the  $\alpha9$  helix and of C-terminus located at the core of virion (table) is noticeable, which confirms availability of channels/cavities in the virion that facilitate partial penetration of tritium atoms (Fig. 1).

Hence, increase of the level of incorporation of the tritium label in the case of removal of the  $\Delta N$ -peptide in the PVA at the elements  $\alpha1$  and  $\beta4$  indicates shielding of the surface domain I and part of the central domain II by the  $\Delta N$ -peptide; and labeling of the  $\alpha6$  helix indicates partial shielding of interhelix region of the surface in the neighboring subunits and localization of

the  $\Delta N$ -peptide above these sites of the CP structure. Decrease of the level of label incorporation inside the virion with deleted  $\Delta N$ -peptide (Fig. 2d) suggests possible structural rearrangement resulting in the virion compaction.

## DISCUSSION

It has been discovered previously that the N-terminal domains of the CP of potyviruses are located at the surface of virions [3, 4, 12] and contain a significant portion of unordered regions [1, 2]. N-terminal peptides of the potyviruses are most immunodominant and generate virus-specific antibodies [32]. In the course of their life cycle potyviruses interact with numerous proteins and membrane components of the plant cell, and availability of a significant fraction of unordered sequences in the CP could be one of the factors explaining their high biological efficiency [33].  $\Delta N$ -domain includes a large number (~70%) of hydrophilic (9 T/S/N/Q/G) and charged (13 E/D/K/R) amino acid residues and a relatively low number of hydrophobic/aliphatic residues (10 I/L/V/A), which defines the protein propensity to structural rearrangements, and could be the reason for abnormal CD spectrum and electrophoretic mobility [1]. It was found out that the  $\Delta N$ -domains of the PVA CP participate in the assembly of virus-like particles (VLP), and part of their unstructured segments acquires  $\beta$ -structure [34, 35].



**Fig. 4.** Relative change of accessible area of CH-surface in the elements of secondary structure in the free protein and in the fragment of virion consisting of 70 CP molecules. Sites with  $(S_{CP\ PVA} - S_{PVA})/S_{CP\ PVA} > 0.43$  are shown in red/yellow. a) Fragment consisting of 4 subunits is shown, b) fragment consisting of one subunit is shown; C, C-end of CP; c, d) 4 subunits outside and inside the virion are shown, respectively; RNA is shown in blue color (a, d).

It has been shown in our study that the  $\Delta N$ -peptide (1-32 aa) shields the residues 66-73, 141-146, as well as residues 161-175 that are in composition of the interhelix region of the PVA CP. Decrease of the level of incorporated label inside the virion with deleted  $\Delta N$ -peptide (Fig. 2d) indicates formation of a more compact helix-shaped viral particle. The observed changes in labeling of the CP fragments after trypsinolysis cannot be caused by elimination of defective virions. This statement is corroborated by the facts of insignificant changes of virus concentration and maintenance of the  $E_{260}/E_{280}$  ratio ( $\sim 1.25$ ) for the virus preparations after trypsinoly-

sis (see Material and Methods). The previously obtained results of analysis of the CD spectra demonstrated that removal of the  $\Delta N$ -peptide did not cause any changes in the secondary structure of the CP in the virion composition. However, the data of synchrotron small angle X-ray scattering (SAXS) and other methods demonstrated changes of the quaternary structure of the PVA virion after removal of the  $\Delta N$ -peptide [5] accompanied by the decrease of the pitch of the protein coat helix, decrease of flexibility, and increase of stability of the particles, which is confirmed by our data. It is known that the spiral shape of the virion structure is in part explained by



the helical shape of the RNA molecule inside the virion, because assembly of the PVY CP in the absence of RNA results in formation of non-helix VLP with stacked-disc architecture [4]. Moreover, CP of poly- and potexviruses [7] interact with RNA at conserved positions (for PVA these include S127, R159, D203). Calculations for the PVY virion (Fig. 3) demonstrated low accessibility of the CH-surface of these residues (0.02–0.09 nm<sup>2</sup>) for the spherical probe with tritium radius; we also observed low radioactivity of these residues both in the intact PVA and in the PVA $\Delta$ 32 (Fig. 1), which provides additional justification for the use of PVY model in our study.

Tritium label was insignificantly incorporated into the inner layers of the virion (Fig. 2d), which suggested migration of reactive tritium atoms through the inter-subunit contacts or channels in the virion filled with aqueous solution. The possibility of such migration has been discussed previously [16, 17]. Tritium atoms can penetrate between the hydrocarbon chains of lipids in liposomes [36] or of purple membranes with bacteriorhodopsin [37] without significant loss of their energy.

A series of biochemical and biophysical studies of icosahedral viruses showed that their capsids are assembled via dynamic self-assembly and are subjected to the controlled conformational transitions changing their surface structures for performing different biological functions [38]. Some helix-shaped viruses demonstrate structural heterogeneity. In particular, ‘wide’ and ‘narrow’ particles with 22.2 and 21.2 subunits per turn were observed among the virions of the barley stripe mosaic virus [39]. A number of studies showed presence of several functional and structural states among the virions of the potato virus X [28, 40, 41]. Analysis of the virions of the TuMV with cryo-EM showed that the virions can extend or shrink with amplitude of approximately 0.22 nm; particles with helix pitch in the range 3.42–3.61 nm were also observed [7]. Removal of the N-terminal peptide from the PVA CP resulted in the decrease of the helix pitch of the virion.

## CONCLUSIONS

It was shown in this study that the N-terminal peptide ( $\Delta$ N-peptide) at the surface of the PVA virion shields the surface domain I, part of the central domain II, and interhelix region at the surface of neighboring subunits. Removal of the  $\Delta$ N-peptide caused decrease in accessibility of the inner region of the virion and resulted in formation of more compact helix-shaped virus particle. We assume that part of the surface  $\Delta$ N-peptide is located between the turns of the virion helix. This increases pitch of the helix and ensures higher flexibility of the virion, which is important for the virus functioning. Understanding of the structural features of flexible phytoviruses and of the role of N-terminal domains in their

coat proteins is necessary for construction and further application of virus particles and virus-like nanoparticles as a platform for presentation of epitopes and vaccine development [42].

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## REFERENCES

1. Ksenofontov, A. L., Paalme, V., Arutyunyan, A. M., Semenyuk, P. I., Fedorova, N. V., Rumvolt, R., Baratova, L. A., Jarvekulg, L., and Dobrov, E. N. (2013) Partially disordered structure in intravirus coat protein of potyvirus potato virus A, *PLoS One*, **8**, e67830, doi: 10.1371/journal.pone.0067830.
2. Charon, J., Theil, S., Nicaise, V., and Michon, T. (2016) Protein intrinsic disorder within the *Potyvirus* genus: from proteome-wide analysis to functional annotation, *Mol. BioSystems*, **12**, 634–652, doi: 10.1039/c5mb00677e.
3. Baratova, L. A., Efimov, A. V., Dobrov, E. N., Fedorova, N. V., Hunt, R., Badun, G. A., Ksenofontov, A. L., Torrance, L., and Jarvekulg, L. (2001) In situ spatial organization of Potato virus A coat protein subunits as assessed by tritium bombardment, *J. Virol.*, **75**, 9696–9702, doi: 10.1128/JVI.75.20.9696-9702.2001.
4. Kezar, A., Kavcic, L., Polak, M., Novacek, J., Gutierrez-Aguirre, I., Znidaric, M. T., Coll, A., Stare, K., Gruden, K., Ravnikar, M., Pahovnik, D., Zagar, E., Merzel, F., Anderluh, G., and Podobnik, M. (2019) Structural basis for the multitasking nature of the potato virus Y coat protein, *Sci. Adv.*, **5**, eaaw3808, doi: 10.1126/sciadv.aaw3808.
5. Shtykova, E. V., Petoukhov, M. V., Fedorova, N. V., Arutyunyan, A. M., Skurat, E. V., Kordyukova, L. V., Moiseenko, A. V., and Ksenofontov, A. L. (2021) The structure of the potato virus A particles elucidated by small angle X-ray scattering and complementary techniques, *Biochemistry (Moscow)*, **86**, 230–240, doi: 10.1134/S0006297921020115.
6. Zamora, M., Mendez-Lopez, E., Agirrezabala, X., Cuesta, R., Lavin, J. L., Sanchez-Pina, M. A., Aranda, M. A., and Valle, M. (2017) Potyvirus virion structure shows con-

- served protein fold and RNA binding site in ssRNA viruses, *Sci. Adv.*, **3**, eaao2182, doi: 10.1126/sciadv.aao2182.
7. Cuesta, R., Yuste-Calvo, C., Gil-Carton, D., Sanchez, F., Ponz, F., and Valle, M. (2019) Structure of Turnip mosaic virus and its viral-like particles, *Sci. Rep.*, **9**, 15396, doi: 10.1038/s41598-019-51823-4.
  8. Agirrezabala, X., Mendez-Lopez, E., Lasso, G., Sanchez-Pina, M. A., Aranda, M., and Valle, M. (2015) The near-atomic cryoEM structure of a flexible filamentous plant virus shows homology of its coat protein with nucleoproteins of animal viruses, *eLife*, **4**, e11795, doi: 10.7554/eLife.11795.
  9. DiMaio, F., Chen, C. C., Yu, X., Frenz, B., Hsu, Y. H., Lin, N. S., and Egelman, E. H. (2015) The molecular basis for flexibility in the flexible filamentous plant viruses, *Nat. Struct. Mol. Biol.*, **22**, 642-644, doi: 10.1038/nsmb.3054.
  10. Tatineni, S., Kovacs, F., and French, R. (2014) Wheat streak mosaic virus infects systemically despite extensive coat protein deletions: identification of virion assembly and cell-to-cell movement determinants, *J. Virol.*, **88**, 1366-1380, doi: 10.1128/JVI.02737-13.
  11. Jarveculg, L., Baratova, L., Dobrov, E., Badun, G., Hunt, R., Andreeva, E., Rabenstein, F., Efimov, A. V. (2000) Study of the spatial structure of potato virus. A coat protein subunits and particles using tritium planigraphy, *Beiträge Züchtungsfors.*, **6**, 61-66.
  12. Shukla, D. D., Thomas, J. E., McKern, N. M., Tracy, S. L., and Ward, C. W. (1988) Coat protein of potyvirus-4. Comparison of biological properties, serological relationships, and coat protein amino acid sequences of four strains of potato virus Y, *Arch. Virol.*, **102**, 207-219, doi: 10.1007/BF01310826.
  13. Atreya, P. L., Lopez-Moya, J. J., Chu, M., Atreya, C. D., and Pirone, T. P. (1995) Mutational analysis of the coat protein N-terminal amino acids involved in potyvirus transmission by aphids, *J. Gen. Virol.*, **76**, 265-270, doi: 10.1099/0022-1317-76-2-265.
  14. Harrison, B. D., and Robinson, D. J. (1988) Molecular variation in vector-borne plant viruses: epidemiological significance, *Philos. Trans. R. Soc. Lond. Ser. B Biol. Sci.*, **321**, 447-462, doi: 10.1098/rstb.1988.0102.
  15. Goldanskii, V. I., Kashirin, I. A., Shishkov, A. V., Baratova, L. A., and Grebenshchikov, N. I. (1988) The use of thermally activated tritium atoms for structural-biological investigations: the topography of the TMV protein-accessible surface of the virus, *J. Mol. Biol.*, **201**, 567-574, doi: 10.1016/0022-2836(88)90638-9.
  16. Baratova, L. A., Bogacheva, E. N., Goldansky, V. I., Kolb, V. A., Spirin, A. S., and Shishkov, A. V. (1999) Tritium planigraphy of biological macromolecules [in Russian], Nauka, Moscow.
  17. Badun, G. A., and Fedoseev, V. M. (2001) Permeability of lipid membranes for atomic tritium or atom "slipping" effect and its role in tritium planigraphy, *Radiochemistry*, **43**, 301-305, doi: 10.1023/A:1012872927896.
  18. Badun, G. A., and Chernysheva, M. G. (2023) Tritium thermal activation method. Features of application, modern achievements, and further development prospects, *Radiochemistry*, **65**, 185-197, doi: 10.1134/S1066362223020054.
  19. Agafonov, D. E., Kolb, V. A., and Spirin, A. S. (1997) Proteins on ribosome surface: measurements of protein exposure by hot tritium bombardment technique, *Proc. Natl. Acad. Sci. USA*, **94**, 12892-12897, doi: 10.1073/pnas.94.24.12892.
  20. Dobrov, E. N., Badun, G. A., Lukashina, E. V., Fedorova, N. V., Ksenofontov, A. L., Fedoseev, V. M., and Baratova, L. A. (2003) Tritium planigraphy comparative structural study of tobacco mosaic virus and its mutant with altered host specificity, *Eur. J. Biochem.*, **270**, 3300-3308, doi: 10.1046/j.1432-1033.2003.03680.x.
  21. Baratova, L. A., Grebenshchikov, N. I., Dobrov, E. N., Gedrovich, A. V., Kashirin, I. A., Shishkov, A. V., Efimov, A. V., Jarveculg, L., Radavsky, Y. L., and Saarma, M. (1992) The organization of potato virus X coat proteins in virus particles studied by tritium planigraphy and model building, *Virology*, **188**, 175-180, doi: 10.1016/0042-6822(92)90747-d.
  22. Shishkov, A. V., Goldanskii, V. I., Baratova, L. A., Fedorova, N. V., Ksenofontov, A. L., Zhirmov, O. P., and Galkin, A. V. (1999) The *in situ* spatial arrangement of the influenza A virus matrix protein M1 assessed by tritium bombardment, *Proc. Natl. Acad. Sci. USA*, **96**, 7827-7830, doi: 10.1073/pnas.96.14.7827.
  23. Ksenofontov, A. L., Kozlovskii, V. S., Kordiukova, L. V., Radiukhin, V. A., Timofeeva, A. V., and Dobrov, E. N. (2006) Determination of concentration and aggregate size in influenza virus preparations using the true UV-absorption spectra [in Russian], *Mol. Biol.*, **40**, 172-179.
  24. Laemmli, U. K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4, *Nature*, **227**, 680-685, doi: 10.1038/227680a0.
  25. Goodman, R. M. (1975) Reconstitution of potato virus X *in vitro*. I. Properties of the dissociated protein structural subunits, *Virology*, **68**, 287-298, doi: 10.1016/0042-6822(75)90272-x.
  26. Tsugita, A., and Scheffler, J. J. (1982) A rapid method for acid hydrolysis of protein with a mixture of trifluoroacetic acid and hydrochloric acid, *Eur. J. Biochem.*, **124**, 585-588, doi: 10.1111/j.1432-1033.1982.tb06634.x.
  27. Trofimova, L., Ksenofontov, A., Mkrtychyan, G., Graf, A., Baratova, L. A., and Bunik, V. I. (2016) Quantification of rat brain amino acids: Analysis of the data consistency, *Curr. Anal. Chem.*, **12**, 349-356, doi: 10.2174/1573411011666151006220356.
  28. Lukashina, E., Ksenofontov, A., Fedorova, N., Badun, G., Mukhamedzhanova, A., Karpova, O., Rodionova, N., Baratova, L., and Dobrov, E. (2012) Analysis of the role of the coat protein N-terminal segment in *Potato virus X* virion stability and functional activity, *Mol. Plant Pathol.*, **13**, 38-45, doi: 10.1111/j.1364-3703.2011.00725.x.

29. Abraham, M. J., Murtola, T., Schulz, R., Páll, S., Smith, J. C., Hess, B., and Lindahl, E. (2015) GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers, *SoftwareX*, **1-2**, 19-25, doi: 10.1016/j.softx.2015.06.001.
30. Gedrovich, A. V., and Badun, G. A. (1992) Study of the spatial structure of globular proteins by tritium planigraphy. Short peptides as a model of a fully extended polypeptide chain [in Russian], *Mol. Biol.*, **26**, 558-564.
31. Lee, B., and Richards, F. M. (1971) The interpretation of protein structures: estimation of static accessibility, *J. Mol. Biol.*, **55**, 379-400, doi: 10.1016/0022-2836(71)90324-x.
32. Shukla, D. D., Tribbick, G., Mason, T. J., Hewish, D. R., Geysen, H. M., and Ward, C. W. (1989) Localization of virus-specific and group-specific epitopes of plant potyviruses by systematic immunochemical analysis of overlapping peptide fragments, *Proc. Natl. Acad. Sci. USA*, **86**, 8192-8196, doi: 10.1073/pnas.86.21.8192.
33. Wei, T., Huang, T. S., McNeil, J., Laliberte, J. F., Hong, J., Nelson, R. S., and Wang, A. (2010) Sequential recruitment of the endoplasmic reticulum and chloroplasts for plant potyvirus replication, *J. Virol.*, **84**, 799-809, doi: 10.1128/JVI.01824-09.
34. Ksenofontov, A. L., Parshina, E. Y., Fedorova, N. V., Arutyunyan, A. M., Rumvolt, R., Paalme, V., Baratova, L. A., Jarvekulg, L., and Dobrov, E. N. (2016) Heating-induced transition of Potyvirus Potato Virus A coat protein into beta-structure, *J. Biomol. Struct. Dynamics*, **34**, 250-258, doi: 10.1080/07391102.2015.1022604.
35. Ksenofontov, A. L., Dobrov, E. N., Fedorova, N. V., Arutyunyan, A. M., Golanikov, A. E., Jarvekulg, L., and Shtykova, E. V. (2018) Structure of potato virus A coat protein particles and their dissociation [in Russian], *Mol. Biol.*, **52**, 1055-1065, doi: 10.1134/S0026898418060101.
36. Kordyukova, L. V., Ksenofontov, A. L., Badun, G. A., Baratova, L. A., and Shishkov, A. V. (2001) Studying liposomes by tritium bombardment, *Biosci. Rep.*, **21**, 711-718, doi: 10.1023/a:1015572321508.
37. Shishkov, A. V., Ksenofontov, A. L., Bogacheva, E. N., Kordyukova, L. V., Badun, G. A., Alekseevsky, A. V., Tsetlin, V. I., and Baratova, L. A. (2002) Studying the spatial organization of membrane proteins by means of tritium stratigraphy: bacteriorhodopsin in purple membrane, *Bioelectrochemistry*, **56**, 147-149, doi: 10.1016/s1567-5394(02)00018-x.
38. Chakravarty, A., Reddy, V. S., and Rao, A. L. N. (2020) Unravelling the stability and capsid dynamics of the three virions of brome mosaic virus assembled autonomously *in vivo*, *J. Virol.*, **94**, doi: 10.1128/JVI.01794-19.
39. Clare, D. K., Pechnikova, E. V., Skurat, E. V., Makarov, V. V., Sokolova, O. S., Solovyev, A. G., and Orlova, E. V. (2015) Novel inter-subunit contacts in barley stripe mosaic virus revealed by cryo-electron microscopy, *Structure*, **23**, 1815-1826, doi: 10.1016/j.str.2015.06.028.
40. Rodionova, N. P., Karpova, O. V., Kozlovsky, S. V., Zayakina, O. V., Arkhipenko, M. V., and Atabekov, J. G. (2003) Linear remodeling of helical virus by movement protein binding, *J. Mol. Biol.*, **333**, 565-572, doi: 10.1016/j.jmb.2003.08.058.
41. Lukashina, E., Badun, G., Fedorova, N., Ksenofontov, A., Nemykh, M., Serebryakova, M., Mukhamedzhanova, A., Karpova, O., Rodionova, N., Baratova, L., and Dobrov, E. (2009) Tritium planigraphy study of structural alterations in the coat protein of *Potato virus X* induced by binding of its triple gene block 1 protein to virions, *FEBS J.*, **276**, 7006-7015, doi: 10.1111/j.1742-4658.2009.07408.x.
42. Steele, J. F. C., Peyret, H., Saunders, K., Castells-Graells, R., Marsian, J., Meshcheriakova, Y., and Lomonosoff, G. P. (2017) Synthetic plant virology for nanobiotechnology and nanomedicine, *Wiley Interdiscip. Rev. Nanomed. Nanobiotechnol.*, **9**, e1447, doi: 10.1002/wnan.1447.

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