Random coil to alpha helix structural shift in the peptide from phospholipase domain of Parvovirus B19 capsid conjugated with the carrier is caused by acidification of the medium

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Abstract

Spectroscopic studies on isolated domains of large proteins and peptides derived from them are complicated because of their tendency to dissolve in aquatic buffers in form of oligomers. Here we demonstrate that conjugation of such peptides with a carrier protein is helpful for studies on the influence of different factors on their secondary structure. Namely, we approved that SK30 peptide from phospholipase A2 domain of Parvovirus B19 capsid conjugated to BSA through N-terminal Cys residue and SMCC linker undergoes random coil to alpha helix transition in the acidic medium mimicking the environment of endolysosome because of His residue protonation. In contrast, unconjugated peptide does not undergo such shift because it forms stable octamers connected by intermolecular beta sheet, as well as the whole isolated phospholipase domain. Obtained results provide evidence on the mechanism of phospholipase domain folding during the acidification of the medium by the way of the formation of contacts between protonated His153 and Asp175, and opens up a perspective of vaccine development, since rabbit polyclonal antibodies against our conjugate, in which the structure of the second alpha helix from the phospholipase A2 domain has been reproduced, can bind epitopes of the complete unique part of VP1 Parvovirus B19 capsid.

Introduction

An evolutionary predecessor of Parvovirus B19 once has stolen the enzyme from its host [1]. Now amino acid sequence that is homologous to eukaryotic phospholipase A2 is inside the long VP1 capsid protein of that virus [2]. And that sequence folds into the enzyme that has catalytic activity necessary for the hydrolysis of phospholipids [3]. Such function is helpful for the virus during the process of the entrance of its genetic material into the cytoplasm from the endolysosome [4]. Inside the endolysosome pH level is around 5.0 [5]. So, one may expect that phospholipase domain of Parvovirus B19 capsid undergoes some structural changes during the acidification of the medium after its fusion with lysosome. Interestingly, circular dichroism (CD) analysis of an isolated synthetic viral phospholipase domain (residues 91–227) did not show any structural shifts associated with changes in pH [6]. On the other hand, catalytic activity of that domain increases drastically with the acidification of the solution [6]. These two controversial facts have been explained in the current study with a shorter fragment of phospholipase domain (residues 144–159 joint by Gly with residues 171–183) that forms oligomers resistant to the decrease of pH, and its conjugate with Bovine serum albumin (BSA) where monomeric peptides do change their structure from coil to alpha helix being protonated.

Protein folding is still a mystery even through numerous predictive methods have been developed [7]. The cause of the mystery is in the fact that polyproteins usually change their structure locally and sometimes even totally during their functioning [8]. Even the most powerful recent 3D modeling methods are able to find just a single snapshot of the most probable and/or thermodynamically favorable structure [9]. However, if one deals with multiple possible structures produced by different methods, then some new data can be obtained about functional structural shifts [10]. Fragments of proteins responsible of such shifts may be revealed with a help of specific software, as well as intrinsically disordered regions [11].
Viral surface proteins usually show high degree of plasticity: their function is to open up the door into the cell for the genetic material of a virus. Recently we found out that receptor binding domain of Parvovirus B19 is able to form intermolecular beta structure at pH = 7.4, and not at pH = 5.0 [10]. In the current study we revealed that phospholipase domain of that virus is not something pH-resistant as well.

Sometimes one can study structural shifts of one of the domains of a viral protein using just a short peptide. However, not each and every peptide model is reliable. In case if a peptide contains hydrophobic amino acid residues, it forms oligomers in aquatic medium: hydrophobic surfaces tend to interact with each other, and so the final structure of the peptide may differ from the expected one. If the peptide contains a lot of hydrophobic residues, it will demonstrate quite low solubility in water that would be not enough to apply any spectral method to estimate the structure. In case if a peptide does not contain hydrophobic residues, in the most of the cases it will not form neither oligomers, nor any concrete structure in water solution [11]. In other words, amphiphilic peptide model may provide some reliable information on structural shifts of a full length protein [10], while hydrophilic peptide model is able to provide some information only on the behavior of intrinsically disordered proteins, and hydrophobic peptides are good for hydrophobic solvents only. In the current study we overcame the problem of oligomer formation by the peptide from parvovirus B19 capsid phospholipase domain by the way of its conjugation with the carrier protein. Moreover, we increased its solubility in water by the reduction of the length of the hydrophobic loop.

Material and Methods

Pre-experimental bioinformatic study

As the material for 3D modeling we used consensus amino acid sequence of phospholipase domain of Parvovirus B19 1a1 subtype VP1 capsid protein (Uniprot: Q9PZT0). Swiss model [12] was used in automatic regime and found a template for homologous modeling (PDB: 1POC) that was bee-venom phospholipase A2.

To check stability of secondary structure elements we used PentaFOLD 3.0 [13] that is propensity based algorithm. To find fragments of the domain prone to certain types of structural shifts and intrinsically disordered regions we used PentUnFOLD server [11].

Experimental spectroscopic study

As the material for the experimental part of the study we used SK30 synthetic peptide (SAVDAARIHDFRYSQGWTVADEELLKNIK) and conjugate of CSK31 peptide (C SAVDAARIHDFRYSQGWTVADEELLKNIK) with bovine serum albumin (BSA) through SMCC linker, as well as BSA itself. Level of purity of those peptides was higher than 95% according to HPLS-MS quality control. Peptides were synthetized commercially (Elabscience) via FMOC protocol.
For the CD spectroscopy we used saturated solutions of SK30 peptide and CSK31 conjugate with BSA in 0.01M phosphate buffers (with pH = 7.4 and pH = 5.0). Undissolved material has been removed from each solution by centrifugation at 5°C with 16000 g. Then CD spectra were recorded on the Chirascan spectrograph in the Shared-Access Equipment Centre "Industrial Biotechnology" of Russian Academy of Science under the following conditions: \( \lambda = 180–280 \text{ nm} \), 1 nm slits, during the heating from 5°C to 50°C. The speed of the heating was 1 K per minute. Concentration of each peptide has been determined using the absorbance value recorded by the same Chirascan spectrograph and extinction coefficient calculated from amino acid sequence [14]. The path length of the cuvette was 0.1 cm. Collected spectra were analyzed by BeStSel server [15].

For the infra-red spectroscopy with multiple horizontal attenuated total reflectance equipment (MHATR-IR) we used the Thermo Nexus 670 FT-IR ESP Nicolet spectrograph. Solutions were prepared in the same way as for CD, but at the room temperature. Excessive water from each drop of the solution (50–100 mcl) was evaporating during 40–50 minutes. Once the spectrum had stopped to change its shape, we collected 5–8 atmosphere corrected and baseline corrected spectra of partially dehydrated polyprotein to build an average one. Additionally, solution of SK30 in 0.01M phosphate buffer at pH = 7.4 has been filtered through 30 kDa and through 10 kDa molecular mass cut off centrifuge filter (Merk) before infra-red spectroscopy.

**Experimental electrophoretic study**

We used modified protocol for blue native gel electrophoresis [10]. As the molecular mass marker we used cytochrome c that exists as a mixture of mono-, di-, tri-, and tetramers giving the bands of 12, 24, 36, and 48 kDa [16]. The sense of blue native gel electrophoresis is to keep protein-protein complexes, including oligomers, but to make them all negatively charged. The way to achieve such goal is to dissolve proteins in a solution containing colloid Coomassie Brilliant Blue G-250. That dye binds proteins and brings them negative charge, but does not cause dissociation of protein-protein complexes. The fact that one may not even need to stain a gel with a dye but just to wash it in the destaining solution is another benefit of that method. We repeated electrophoresis several times in buffers with pH = 7.4 and pH = 5.0 (50 mM tricine, 15 mM BIS-TRIS, and 0.02% Coomassie Brilliant Blue G-250 solution as the cathode buffer with pH = 7.4 or pH = 5.0; 50 mM BIS-TRIS with pH = 7.4 or pH = 5.0 as the anode buffer).

**Experimental immunological study**

We immunized two rabbits with SK30 peptide and two other rabbits with CSK31 peptide conjugate with BSA. Experiments on animals have been approved by the Biomedical Ethics Committee of Belarusian State Medical University (protocol №7, 27.12.2021). The protocol included initial intradermal injection (in 8 sites of the rabbit body) of the antigen (1 mg) in complete Freud adjuvant and three following injections (1 mg) of the same antigen in incomplete Freud adjuvant. Pauses between injections were equal to two weeks. Blood was taken a day before the first immunization and a week after the last immunization.

To collect antibodies, we used affine chromatography with SK30 peptide (1 mg) immobilized on AminoLink Plus column and with CSK31 conjugate with BSA immobilized on SulfoLink column. It is
important to notice that SK30 was dissolved in the buffer with pH = 10 for the initial step of immobilization. The presence of immunoglobulin in eluates was checked with fluorimeter Solar 2203. Excitation wavelength was equal to 280 nm. Emission spectra (300–400 nm) were recorded with a step of 1 nm.

Antigen-antibody complexes are destroyed in the elution buffer that is 0.1M glycine HCl buffer with pH = 2.5 according to the standard affine chromatography protocol [17]. Interestingly, such elution works good if a serum was applied to the column. However, when solution of a pure antibody or antigen is applied to such column, stronger complexes are formed that require the heating at 60°C for 10 minutes to be destroyed [13]. After such heating in acidic medium immunoglobulins and other proteins can become denatured and may show fluorescence spectra with maximum at 304 nm, indicating the absence of energy transfer from Tyr to Trp residues [18].

Additional affine chromatography experiments included immobilization of recombinant VP1u protein on NHS-activated agarose column and checking the ability of purified antibodies against CSK31 conjugate with BSA to bind it; immobilization of purified antibodies against CSK31 conjugate with BSA on such column and checking the ability of recombinant VP1u protein to bind them; checking the ability of purified antibodies against CSK31 conjugate with BSA to bind immobilized SK30 peptide.

Post-experimental bioinformatic study

To model the structure of SK30 peptide we used QUARK software [19]. Then the best model was optimized by the GalaxyRefine2 server [20]. 3D structures were visualized by RasMol. To find a model for SK30 peptide in the relaxed state we used APPTEST server [21] that performed molecular dynamics for the best QUARK model of the peptide.

Results

3D model of phospholipase domain

The model of Parvovirus B19 phospholipase domain has been built with a help of SwissModel server based on a template of phospholipase A2. Such model represents typical architecture of phospholipase A2 [22] that features three alpha helices and a long relatively hydrophobic loop between the first and the second alpha helices (Fig. 1a). That loop has been shown to dig into the membrane letting the second ester bond of phospholipids go straight into the active center of the enzyme [4].

To check stability of phospholipase domain we used PentUnFOLD server [11]. According to the results of PentUnFOLD 1D version that considers just amino acid sequence, the only stable element of secondary structure for the whole domain is the second alpha helix. However, 2D version of the same server that considers the information on secondary structure, described the first alpha helix as a stable one together with the second alpha helix (see supplementary Material, Table S1). Interestingly, PentUnFOLD 3D
considered only the first alpha helix as a fragment that cannot turn into disordered state. As one can see in Fig. 1, the first and the second helices form numerous contacts with each other.

The PentaFOLD 3.0 algorithm [13] predicted that both helices are stable enough, unlike the third metastable one (Fig. 1b). To decrease hydrophobicity of the peptide we reduced the length of the long loop between the first and the second alpha helices. To decrease the level of noise in spectral data we also deleted unstable third alpha helix. As a result, we concentrated our attention on interactions between the first and the second alpha helices of phospholipase A2 domain. According to our hypothesis, the second alpha helix is responsible for the formation of the first alpha helix due to numerous interactions, and the shift from random coil to alpha helix may be a mechanism of phospholipase domain activation in the acidic environment. So, we used the synthetic peptide named SK30 in our experiments, as well as the conjugate of that peptide with BSA through additional N-terminal cysteine residue and SMCC linker.

Circular dichroism of SK30 peptide and CSK31 peptide conjugate with BSA

The shape of CD spectra for SK30 peptide solution in 0.01M phosphate buffer is almost the same both at pH = 7.4 and pH = 5.0. Moreover, that shape of spectra recorded by us is quite similar to the known one but for the complete phospholipase A2 domain [6]. In our experiment with just two fragments of that domain included in SK30 peptide the structure was represented by alpha helix (~ 12%) and beta sheet (~ 20%) according to the analysis with BeStSel server [15]. In the previous experiment with the complete domain, percentage of structured regions (~ 10% for alpha helices and ~ 25% for beta sheet) was also quite low [6]. So, the model (Fig. 1) does not correspond to the observed shape of the domain and the peptide. Indeed, the minimum at 202 nm on those spectra (Fig. 2) is rather indicating high percent of random coil than the presence of expected alpha helices [23]. According to our hypothesis, beta structure should be intermolecular in oligomers of both the domain and the peptide. No shifts in secondary structure content caused by the change in pH or the heating were detected by us for the SK30 peptide.

In contrast, spectra of the CSK31 peptide conjugate with BSA are different at pH = 7.4 (Fig. 3a) and pH = 5.0 (Fig. 3b). Even through at both pH values the minimum at 208–222 nm indicates the alpha-helical conformation of the polypeptide [23], at pH = 5.0 (Fig. 3b) that minimum is flatter (does not contain such a sharp minimum at 208 nm), while the maximum at 190 nm is about two times higher than that at pH = 7.4 (Fig. 3a). These features of those spectra have been interpreted by the BeStSel server [15] as follows: at pH = 7.4 the percent of residues in alpha helix is about 30%, but at pH = 5.0 the percent of residues in alpha helix is about 40%. Such random coil to alpha helix shift is caused by the change in conformation of the peptide, and not the carrier protein, since BSA itself has previously been shown to lose its alpha-helical conformation with the acidification of the medium in the CD study: from 75% at pH = 7.0 to 50% at pH = 4.0 [24].

Taken together, conjugation of the SK30 peptide through N-terminal cysteine and SMCC linker prevented formation of oligomers and the most of intermolecular beta sheet. That is why conjugation to a carrier can help to reveal structural shifts characteristic to full length protein.
Infra-red spectroscopy and membrane filtration of SK30 peptide and CSK31 peptide conjugate with BSA

Infra-red spectra of SK30 and CSK31 conjugate with BSA have been recorded with multiple horizontal attenuated total reflectance accessory. Because of this, we are able to check the secondary structure of partially dehydrated polypeptides. As one can see in Fig. 4, at both pH = 7.4 and pH = 5.0 SK30 peptide spectra show two peaks of the amide I band: the first one corresponds to intermolecular beta sheet (1625 cm\(^{-1}\)) [25], the second one corresponds to alpha helix (1656 cm\(^{-1}\)) [25]. For the CSK31 conjugate with BSA one can observe just a single peak at 1656 cm\(^{-1}\) corresponding to alpha helix at both pH values, as well as for BSA itself. Only residual beta structural peak at 1625 cm\(^{-1}\) can be observed in those spectra, and it is especially prominent at pH = 5.0 (Fig. 4). Filtration of SK30 solution through the centrifuge filter with 10 kDa molecular mass cut off has shown that together with bigger oligomers connected by intermolecular beta sheet there are dimers that contain no beta structure and show just a single peak at 1658 cm\(^{-1}\). Interestingly, solution that passed through the filter with 30 kDa molecular mass cut off demonstrated both alpha-helical and beta-structural peaks (Fig. 4).

Gel electrophoresis of SK30 peptide

Blue native gel electrophoresis results confirmed the presence of the major fraction of rather large oligomers of SK30 peptide in a solution along with a minor fraction of smaller oligomers. Results were almost identical at both pH = 7.4 (Fig. 5) and pH = 5.0 in all replica with different electrophoresis chambers and staining methods. Namely, the major band of SK30 is situated between the second and the third bands of cytochrome c, while the minor band of SK30 is under the first band of cytochrome c that was used as a molecular weight marker. Since molecular weight of cytochrome c monomer is 12 kDa, the major oligomer of SK30 peptide has a mass between 24 and 36 kDa. Taking into account molecular mass of SK30 monomer (3.42 kDa), one can suggest that abovementioned synthetic peptide prefers to dissolve in water solution in form of octamers (3.42\times8 = 27.36 kDa). Such octamers indeed should pass freely through the filter with 40 kDa molecular mass cut off, but should retain on a filter with 10 kDa molecular mass cut off.

Immunological confirmation of CSK31 conjugate reliability

We immunized two rabbits by SK30 peptide, and two other rabbits by CSK31 conjugate with BSA according to the standard protocol for polyclonal antibodies production [26]. Antibodies against SK30 were collected using AminoLink column for affine chromatography with immobilized SK30 peptide. Antibodies against CSK31 conjugate with BSA were collected using SulfoLink column with immobilized conjugate. Quantitatively, humoral immune response to the conjugate was stronger than that against the peptide (Fig. 6). Polyclonal antibodies against CSK31 conjugate with BSA have been checked for their ability to bind immobilized SK30 peptide and recombinant VP1u domain of the VP1 capsid protein of Parvovirus B19 with a help of affine chromatography. Some part of those antibodies were able to form
complexes with SK30 peptide and with the full length recombinant VP1u that were not destroyed even at low pH of 2.5 of the IgG elution buffer. Existence of such stable complexes was confirmed by the reverse experiments as well, when collected filtered antibodies against CSK31 conjugate with BSA had been immobilized, while solution of VP1u was applied on those columns. In those affine chromatography experiments (Fig. 7) antigen-antibody complexes were broken after the heating of the column during 10 minutes at the temperature of 60°C only. Antibodies against CSK31 have been eluted from the column with immobilized SK30 peptide mostly after the second turn of the heating, and the shape of the fluorescence spectrum was characteristic for native IgG (with maximum of emission at 343 nm). Same antibodies have been eluted from the column with immobilized recombinant VP1u mostly after the first turn of the heating, but the maximum on a spectrum was at 308 nm, that is the evidence of the denaturation of IgG and the break of the energy transfer from Tyr to Trp residues (Fig. 7a). Recombinant VP1u protein has been eluted from the column with immobilized antibodies against CSK31 mostly in the third eluate, and there was maximum at 308 nm on that spectrum (Fig. 7a). However, the differential spectrum between the third and the second eluates from the same experiment has the shape characteristic to the native (not denatured) recombinant VP1u with the maximum at 343 nm (Fig. 7b). It means that some part of VP1u has been denatured, while another part has not.

The data described in this section confirm that some epitopes of SK30 peptide have been reproduced in the CSK31 conjugate with BSA, as well as that some epitopes of full length VP1u are the same as those of SK30 and CSK31 conjugate with BSA. It is likely that those common epitopes correspond to the second (stable) alpha helix of phospholipase domain, while the rest of the sequence of SK30 peptide might be involved in intermolecular beta sheet that is hidden inside oligomers.

Discussion

Possible pH-sensor that helps phospholipase A2 domain to fold correctly

Experimental data collected in this study can be summarized by the selection of appropriate computer models. Molecular dynamics with the APPTEST server [21] among others produced the structure with random coil (actually, with irregular turns) in the N-terminal half and alpha helix in the C-terminal half without contacts between those two halves of the peptide (Fig. 8a). Refinement with GalaxyRefine2 server [20] of the SK30 peptide model made by QUARK [19] produced the result represented in Fig. 8b. Here two alpha helices are tightly packed and one of their contacts is formed between His10 (His153 in the VP1 protein) and Asp22 (Asp175 in the VP1 protein) residues. Formation of this bond is quite expectable if imidazole nitrogen atom of His is protonated and thus possessing a positive charge [27]. His residues are usually used as pH-sensors in proteins [28], since pK_a value for them is around 6. In the sequence of SK30 peptide there is just a single His residue. It is likely that acidification of the medium causing His residue protonation first brings positive charge to His that should attract the first alpha helix to the second one with negatively charged Asp, and then provides an opportunity for hydrogen bond formation.
Other contacts between amino acid residues are formed as well shifting the equilibrium towards alpha-helical conformation for the N-terminal half of the peptide. So, at pH = 7.4 after the initial abortive contact between viral capsid and cellular receptors phospholipase domain should become exposed to the solvent due to capsid rearrangement [29]. In that state the domain is rather unfolded but the second alpha helix remains (Fig. 8a). Probably, exactly in that state phospholipase domain becomes a target for neutralizing antibodies development [29]. In our opinion, according to the obtained data, phospholipase A2 domain folds into appropriate alpha-helical enzyme (Fig. 8b) only in the endolysosome where it starts to hydrolyze phospholipids. Indeed, enzymatic activity of Parvovirus B19 phospholipase domain is several fold higher at pH = 5.0 than at pH = 7.0 [6]. In the model of phospholipase A2 domain (Fig. 1) the distance between carboxylic oxygen of Asp175 and imidazole nitrogen of His153 is equal to just 2.67 Angstrom. It means that such contact is necessary for the correct folding of phospholipase A2 domain. Moreover, site-directed mutagenesis study [31] showed that either His153 or Asp175 substitution by Ala leads to the almost complete lack of enzymatic activity. Indeed, those residues are thought to form a catalytic center of the enzyme and to form a complex with Ca\textsuperscript{2+} cation [31]. So, His153 and Asp175 must be close enough to each other in the active state of phospholipase A2 domain, and such conformation seems to be formed when both alpha helices are making multiple contacts with each other at acidic pH of the endolysosome. Indeed, phospholipase A2 activity is required after the virion trapped into the endolysosome, and not before the binding with appropriate cellular receptor.

**Perspectives of synthetic vaccine development against Parvovirus B19**

Those antibodies that form such complexes with phospholipase A2 domain that are still stable at acidic pH really must be neutralizing, since they prevent correct folding of the domain and diminish its enzymatic activity. Virions covered by such antibodies have a small chance to escape from endolysosome. From this point of view, CSK31 conjugate with BSA may be considered as a candidate for vaccine development against Parvovirus B19. Such vaccine should have decreased chance to cause antiphospholipid syndrome [32], since the antigen lacks hydrophobic loop of phospholipase domain. As a result of numerous experiments we finally approved that one of the targets for the development of neutralizing antibodies against Parvovirus B19 is the second alpha helix of phospholipase A2 domain.

**Declarations**

**Author Contribution**

VVK and OVK analyzed obtained data and wrote the main manuscript text; ANS reviewed and edited the text; OEB, AVP, VVK, TAK and VVP collected CD spectra; VIC, EGS, AAA and VVK collected MHATR-IR spectra; NVS, ANS, EOS, MAY, GVS, AAA, VVP, VK and OVK performed electrophoresis; AAA and VVK performed centrifugal ultrafiltration; AAA, TAK, BVR, VVK and OVK collected fluorescence spectra; AAA,
UVK, VVP, TAK and VVK immunized rabbits; VVP, VVK and OVK performed affine chromatography; VVP and VVK performed molecular modeling and structure stability analyses.

References


Figures

![Figure 1](image_url)
The model of phospholipase A2 domain from VP1 capsid protein of Parvovirus B19. In panel A residues are colored in the rainbow style: N-terminus is violet, while C-terminus is red, so the first alpha helix is green, the second alpha helix is yellow, the third alpha helix is red. In panel B residues are colored by the PentaFOLD 3.0 algorithm according to the stability of secondary structure element they form: stable residues are green, metastable residues are yellow, unstable residues are red.

![Circular dichroism (CD) spectra](image)

**Figure 2**

Circular dichroism (CD) spectra of SK30 peptide from the phospholipase A2 domain of Parvovirus B19 VP1 capsid protein dissolved in 0.01 M phosphate buffer with pH=7.4 (panel A) and 0.01 M phosphate buffer with pH=5.0 (panel B) during the heating from 5°C till 50°C.
Figure 3

Circular dichroism (CD) spectra of the SMCC conjugate of CSK31 peptide from the phospholipase A2 domain of Parvovirus B19 VP1 capsid protein and Bovine serum albumin (BSA) dissolved in 0.01 M phosphate buffer with pH=7.4 (panel A) and 0.01 M phosphate buffer with pH=5.0 (panel B) during the heating from 5°C till 50°C.
Figure 4

Infra-red spectra recorded with multiple horizontal attenuated total reflectance tool for partially dehydrated solutions of SK30 peptide from the phospholipase A2 domain of Parvovirus B19 VP1 capsid protein; SK30 peptide from the phospholipase A2 domain of Parvovirus B19 VP1 capsid protein; BSA; fraction of SK30 solution passed through the filter with 30 kDa molecular weight cut off; fraction of SK30 solution passed through the filter with 10 kDa molecular weight cut off. Saturated solutions of peptides and proteins in 0.01 M phosphate buffer with pH=7.4 and pH=5.0 were used. Stable spectra after the drying of those samples were recorded.
Electrophoregram for the SK30 peptide and cytochrome c (used as molecular weight marker) obtained with the blue native gel electrophoresis modified protocol.
Figure 6

Results of immunization with SK30 peptide and CSK31 peptide conjugate with BSA. Fluorescence signals proportional to protein concentration are shown for elution fractions (2 ml each) in affine chromatography experiments. For each rabbit results are shown for nonimmune serum and immune serum.
Figure 7

Fluorescence spectra for eluates with the highest signal obtained in affinity chromatography experiments (A) with (i) immobilized SK30 peptide and polyclonal rabbit antibodies against CSK31 peptide conjugate with BSA in mobile phase, (ii) immobilized recombinant VP1u and polyclonal rabbit antibodies against CSK31 conjugate with BSA in mobile phase, (iii) with immobilized polyclonal rabbit antibodies against CSK31 peptide conjugate with BSA and recombinant VP1u in the mobile phase, as well as the differential spectrum between eluates with the highest and the lowest fluorescence signal from the affinity chromatography experiment (B) with immobilized polyclonal rabbit antibodies against CSK31 peptide conjugate with BSA and recombinant VP1u in the mobile phase.
Figure 8

The model of SK30 peptide with unfolded first alpha helix and remaining second alpha helix (A), and the model of SK30 peptide with two alpha helices interacting with each other via His10 and Asp22 along with other contacts. Residues are colored in the rainbow style: N-terminus is violet, while C-terminus is red, so the first alpha helix is blue, the second alpha helix is red.

Supplementary Files

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- TableS1.docx