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Peptide from NSP7 is able to form amyloid-like fibrils: Artifact or challenge to drug design?

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ABSTRACT

We found potential amyloidogenic fragment in NSP7 SARS-CoV2 protein in silico
NSP7 (52–62) fragment is able to form amyloid-like fibrils
The possibility of using such a peptide as the basis for an antiviral drug is discussed

One of the currently frequently discussed methods of antivirals design is the specific induction of changes in the structure and functionality of proteins by a prion-like mechanism [1]. Indeed, viral proteins tend to form amyloid-like fibrils [2], and at the same time, they often have low homology with human proteins. For most proteins, the induction of a conformational transition by peptides is a process that depends on the coincidence of the primary structure of the protein and the peptide acting on it [3]. Together with the ability to amyloid chain reaction, this suggests that such peptides will be effective at low concentrations and, at the same time, will not affect host proteins. A number of peptides are known to have antiviral activity and act according to this mechanism [4,5]. The general strategy for the search for such drugs is the search for an amyloidogenic protein determinant and the design of a peptide that carries this determinant. Such a peptide can specifically affect the parental protein at low concentrations, causing its conformational transition and loss of activity [6]. At the same time, it must be kept in mind that the effect of amyloid-like viral structures on the host has not been studied enough [7] however, for example, in the case of the influenza virus, the formation of amyloid-like fibrils by the PB1-F2 protein during the life cycle of the virus does not have a significant effect on the host organism [8]. The NSP7 protein is a processivity subunit of SARS-CoV-2 RNA-dependent RNA polymerase [9]. In this work, we analyzed the primary structure of NSP7 and proposed sequences of the peptide capable of forming amyloid-like fibrils and having the potential to influence the conformation of this subunit through a prion-like mechanism.

We analyzed the primary structure of the NSP7 protein using three programs – Arches, which allows predicting the formation of hairpins characteristic of amyloid-like proteins [10], FoldAmyloid [11], which analyzes the local amino acid composition, and an original program for searching for mirror symmetry motifs [12]. The choice of these programs was due to the fact that in the course of our previous studies, the programs separately made it possible to predict amyloidogenic peptides. Protein sequence of NSP7 (P0DTD1) from the SWISSPROT database were used. Fig. S1 (A) shows the results of the search for potential amyloidogenic regions in NSP7. The peptide corresponding to the NSP7 sequence from S3 to 61 amino acid residues was determined as amyloidogenic by all three programs. Peptides PINP73 (MVSLLSVLLSM, 1191.66 Da) corresponding to NSP7 potentially amyloidogenic region to 52–62 amino acid residues (comprising 2 symmetrical methionines at the N- and C-terminus) and PINP74 (predicted only by FoldAmyloid and Arches, corresponding to 27–37 NSP amino acid residues, KLWAQCVQ, 975.17 Da) was chemically synthesized at OOO NPF VERTA, Russia, purity more than 80%. Peptides were dissolved in 5 μL of DMSO an then phosphate buffered saline (PBS) buffer was added to the 1 mg/mL peptide concentration (0.5% DMSO), then solutions were incubated for 1 h with agitation at orbital shaker (Eppendorf, USA) at 55 °C, 600 rpm. Obtained PINP73 or PINP74 peptide samples were diluted 70 times with...
water, after which 10 μL of solution were applied onto a freshly cleaved mica substrate. After 1 min of incubation, the sample was dried in compressed air. Images (topography of the sample surface) were obtained in the semi-contact mode on an atomic force microscope “NT-MDT” (NT-MDT, Russia), with NSG01 probe. On the mica surface, filaments similar in morphology to amyloid-like fibrils (about 3 nm in height) were observed for PINP73 (Fig. 1), not for PINP74 peptide sample (Fig. S2). PINP73 fibrils were observed using atomic force microscopy of three independent suspension preparations, and were not observed when analyzing the PINP74 suspension. Image processing was performed using “Gwyddion” software.

In order to determine the amyloid-like nature of the observed filaments, we performed fluorimetry in the presence of Thioflavin T. As a control sample, we used a peptide PINP74 which has a similar to PINP73 molecular weight. Aliquots (30 μL) of peptide solutions were added to 970 μL of 10 mM ThT solution in PBS buffer. Measurements were performed in a HITACHI F-4010 fluorescence spectrophotometer (Hitachi, Japan) with excitation wavelength 440 nm (bandpass 5 nm) and emission wavelength 478 nm (bandpass 5 nm). An increase in Thioflavin T fluorescence was observed in the presence of PINP73, but not PINP74 (Spectra are shown in Fig. S1 (B)) indicates amyloid nature of PINP73 filaments observed by means of atomic force microscopy. Congo Red assay was also performed. Congo Red dye (Sigma Aldrich, USA) at a concentration of 50 μM in PBS was mixed with the 10 μM peptide solution. As a control, a similar sample was used in which a buffer was added instead of the peptide. Absorption spectra were recorded on a BMG Clariostar (BMG, Germany), and difference spectrum was analyzed. Similarly, a comparison of the absorption spectrum of Congo red with PINP74 or PINP73 peptide showed the presence of a peak in the Congo Red with PINP73 spectrum in the region of 550 nm which is characteristic for amyloid-like fibrils (Fig. S1 (C), peak is shown in difference spectrum).

There have been previous attempts to predict the amyloidogenic regions of SARS-CoV-2 proteins. Gour et al. [6] analyzed the known SARS-CoV2 proteins for the presence of amyloidogenic regions. In the cited work, theoretical prediction was performed using the FoldAmyloid, Waltz, and AGGRESCAN software. Interestingly, the PINP74 peptide is located in the region predicted by the FoldAmyloid as amyloidogenic, but does not exhibit amyloidogenicity. Peptide PINP73 is also defined by FoldAmyloid as amyloidogenic, but it also falls within the arches region predicted by the Arches software and is a part of a mirror-symmetric motif. It cannot be said with certainty that the use of these three programs (Arches, FoldAmyloid, and symmetry search) can reliably predict amyloidogenic peptides derived from the whole protein, however, in this work, such an approach made it possible to detect an amyloidogenic peptide. The combination of beta-turn forming prioness, symmetry, and context of amino acid residues may be the key to predicting the ability to form amyloid-like fibrils. In the light of recently appearing in databases of fibril structures obtained using cryo-electron microscopy, such a relationship between the features of the primary structure and the ability to homooligomerization is the subject of our further research. It should be noted that the specified fragment of NSP7 is located in the core part of the protein (Fig. S3). This part is not involved in the formation of a dimer, while the fragment that did not show the ability to form fibrils (27–37) is located in the region of the terminal helix involved in dimerisation [13]. It should be noted that the potentially fibrillogenic region of NSP7 is available for interaction both in the monomer and in the dimer. It is possible that the interactions leading to the formation of higher-order oligomers described in [13] are due to interactions between such protein regions. With regard to viruses, in particular SARS-CoV2, the ability of its proteins and protein fragments to amyloidogenesis is unlikely to be an artifact. Interestingly, for the S-protein, the ability of its fragment to form amyloid-like fibrils was confirmed experimentally [14]. Also, the ability of SARS-CoV2 proteins to form amyloid-like fibrils is considered by a number of researchers as one of the possible pathogenicity factors [7,15].

In this work, we demonstrate for the first time the ability of the NSP7 fragment to form amyloid-like fibrils in vitro. Further studies will be aimed at studying the ability of fibrils formed by this peptide to induce coaggregation of the full-length recombinant NSP7 protein and to study the ability of the peptide to act as an antiviral agent in a cell culture model.

Author statement

VVE – writing the manuscript, spectroscopy methods, sequence analysis; YPG – Atomic force microscopy; AAR – sequence analysis, writing the manuscript.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
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Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1155/2015/723186.

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