

Structural features of the ionic self-complementary amyloidogenic peptide

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Abstract. In this study we investigate the effect of triazavirine on ionic self-complementary (iSCM) containing peptides fibrils with transmission electron microscopy (TEM), small-angle neutron scattering (SANS) and MALDI mass-spectrometry (MS). It was shown that triazavirine is capable to dissociate iSCM amyloid fibrils. The mechanism of such an action is proposed.

1. Introduction

Conformational diseases such as amyloidoses are one of the main causes of the self-care capacity loss in the elderly. Amyloid fibril formation is the process which underlies the pathogenesis of many conformational diseases. Such fibrils have abnormal resistance to enzymes, chemical and physical influences [1].

In this study we investigate the effect of guanine nucleotide analog triazavirine [2] (Figure 4A) on the peptide GDIRIDIRIDIRG (Self-complementary Ionic, SI) fibrils dissociation. SI peptide comprises an ionic self-complementary motif (iSCM), first identified in the study of small heat shock proteins. Interaction between these proteins subunits is provided with antiparallel oriented monomer's iSCMs. It is also shown that iSCM is also involved in the assembly of crystallin oligomeric complexes – the proteins found in various organs, including the eye tissues [3]. SCM – containing peptide fibrils are subjects of nanostructured materials studies. The two-staged mechanism is proposed for iSCM amyloid fibrils formation: electrostatic attraction of protofibrils-forming monomers at the first stage and zipper-like hydrophobic interaction between protofibrils at the second mature fibrils forming stage [4], [5].

From molecular modeling of various proteins interaction with a number of model molecules – such as structural nucleotide analogs, we found that, within the model used, these substances have a tendency to interact with side chains of basic amino acid residues. This fact agrees with NMR guanosine-arginine interaction data [6]. Molecules with such a specificity of interaction may be useful for some disease treatment or prevention [7], [8].

Beta-strands are the main structures involved in the amyloid fibrils formation. Arginine-containing beta-strands are stabilized in amyloid-like fibril with hydrogen bonds and electrostatic attraction. The



selective blocking of arginine residues ionic interaction from one peptide with negatively charged, i.e. aspartate, residues of another could lead to monomer repulsion, hydrogen bonds breakage and fibrils dissociation. This study is dedicated to proving the guanosine analogue triazavirine ability to dissociate amyloid-like fibrils formed with iSCM containing peptide.

2. Materials and methods

2.1. Materials

Peptide GDIRIDIRIDIRG (SI) was synthesized at NPF “Verta” Ltd., purity higher than 90%. All reagents were purchased from Sigma Aldrich, Triazavirine (2-methylthio-6-nitro-1,2,4-triazolo[5,1-c]-1,2,4-triazine-7(4I)-one) substance was a kindly gift from Ural Federal University.

2.2. Transmission electron microscopy (TEM)

TEM was performed using the standard negative-staining technique as described previously [9].

2.3. Small-angle neutron scattering (SANS)

SANS spectra were recorded with a YuMO spectrometer located in the fourth channel of the high-flux IBR-2 reactor (Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna). Measurements were performed in the standard geometry. A neutron beam was collimated into a 14 mm spot. The SANS data were collected on the YuMO spectrometer using two detectors [10]. The sample-detector distances were 12.96 m and 5.3 m for “New” and “OLD” detectors, respectively. Solutions of samples in D₂O buffer were measured at 20°C in 1 mm path-length quartz Hellma cells. The temperature was controlled by Lauda thermostat in special thermobox [11]. The spectrometer during the experiment was governed by SONIX+ Software program [12]. Using SAS program [13] we (i) combined the data referring to the same sample, (ii) calculated the spectrometer resolution function for given experimental conditions, (iii) carried out data correction on dead times of neutron ring wires detectors, (iv) subtracted a background signal from detector data, (v) carried out the normalization of the obtained spectrum on standard vanadium scatterer, and (vi) subtracted background sample.

2.4. Mass-spectrometry

Mass-spectrometry was performed in MALDI TOF “UltrafleXtreme” mass-spectrometer, Bruker, at positive ions registration mode using DHB matrix. Before the sample preparation samples were centrifuged at 1000 g for 10 minutes.

2.5. Fibrils preparations

SI peptide was dissolved in phosphate-buffered saline buffer pH 7.4 to the 5 mg/ml concentration and then incubated at room temperature for 1 hour with agitation. Triazavirine was added at 5x molar excess to the peptide and then the mixture was incubated for an hour at room temperature.

2.6. Molecular docking

Protein docking was performed using the Hex server software (<http://hex.loria.fr/>) with default parameters (electrostatic interactions included).

3. Results and discussion

The model peptide SI was studied by us previously [14]. It was found that it has all properties that characterize amyloidogenic peptides and, at the same time, is simple for computer modelling and studying of its spatial structure and oligomerization using biophysical and biochemical methods. Furthermore, the presence of only four different amino acid residues in the peptide allows us to make targeted influence on non-covalent bonds which drive protein-protein interactions in abnormal fibrils. This work aims at study the influence of the guanidine nucleotide analogue (triazavirine, TZV) on the model peptide fibrillogenesis.

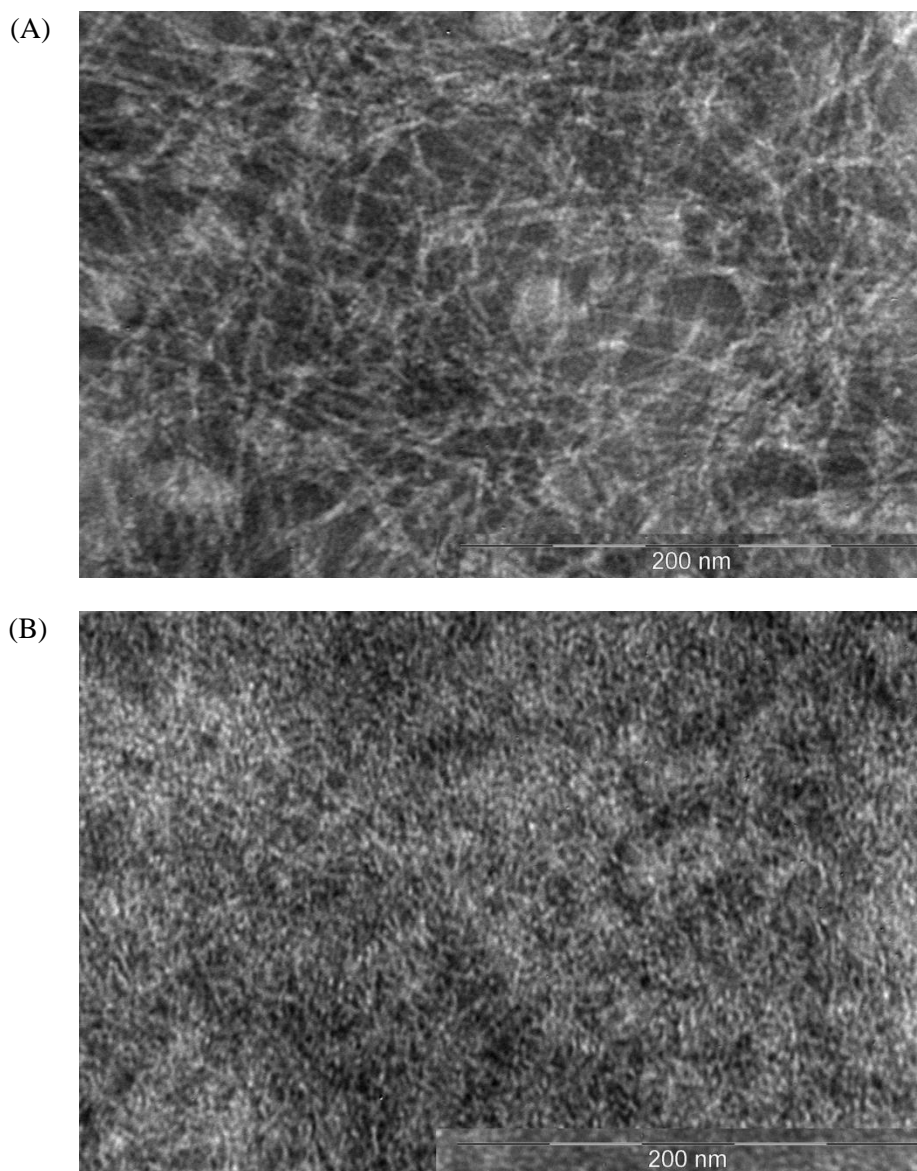


Figure 1. Transmission electron microscopy images of (A) – SI fibrils; (B) – SI fibrils with triazavirine.

As it is obvious from TEM images (Figure 1), TZV administration to the sample of SI fibrils causes shortening of fibrils and partial fragmentation of the fibrils network compared to fibrils in control conditions. Precise and reliable analysis of TZV-dependent SI fibrils structure remodeling requires additional methods in combination with TEM. Also, sorption of different species in a polydisperse sample (fibrils of different morphology, oligomers and unstructured aggregates) to the TEM grid surface may differ and could be further affected by the presence of nucleotide analog. Therefore to obtain the structural information on all of the species present in the bulk sample and for the further structure refinement we made additional experiments for SANS spectra measurements.

The SANS spectra presented in Figure 2 show substantial scattering from SI that generally followed a power law with exponent close to -2 , indicating that significant SI peptide fraction forms large random-coil linear oligomeric structures or unstructured aggregates. Scattering by peptide alone was in agreement with a model of long worm-like fibrils with the diameter of approximately 3 nm and Kuhn length of ca. 12 nm (Figure 2A). The fibril diameter yielded by this approximation is close to the

size of SI tetramers proposed earlier as the main prefibrillar species comprising fibrils of SI peptide [14].

In contrast with SI alone, scattering of the peptide after TZV was added to the sample could not be adequately described by worm-like model, but rather by a mixture of Gaussian coil polymer and rod-like fibrils approximately 10 nm in diameter (Figure 2B).

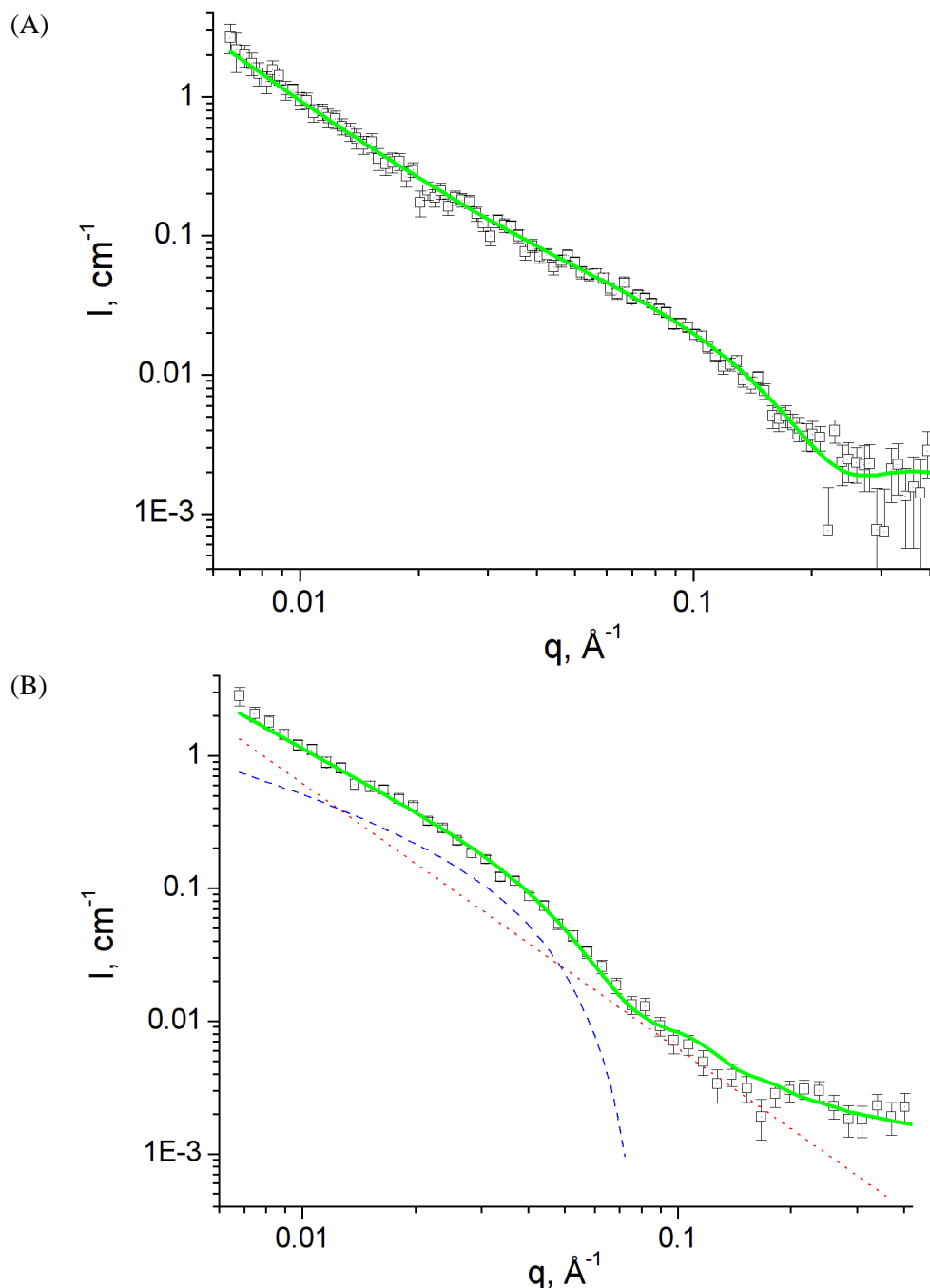


Figure 2. Small-angle neutron scattering curves analysis results of (A) – SI fibrils; (B) – SI fibrils with triazavirine. Data fitted to (A) worm-like model with fibril radius of 1.40 ± 0.04 nm and Kuhn length of 12.0 ± 0.7 nm ($\chi^2 = 0.83$, solid green line in Panel A) and (B) linear combination of random coil model (dotted red line) and long cylinders with the radii of 4.66 ± 0.14 nm (dashed blue line), $\chi^2 = 1.2$, shown in solid green line in Panel B.

The samples were further separated by centrifugation and MALDI mass-spectrometry (MS) measurement of pellets and supernatants was performed. The presence of signal in spectrum at Figure 3, A (SI peptide fibrils before centrifugation) shows that SI peptide molecules are capable for ionization and can be detected by MALDI with the matrix chosen. The analysis showed that in SI fibrils sample there is no detectable quantity of the peptide complexes in supernatant (Figure 3B) with all peptide molecules being in the pellet (Figure 3C). After addition of triazavirine we observed no pellet in the same centrifugation conditions, but the signal which corresponding to SI peptide was detected in the supernatant (Figure 3D).

Taken together, the TEM, SANS and MS data indicate that SI fibrils were partially dissociated after triazavirine addition. In solution nearly all of the peptide appear to form long worm-like fibrils ca. 3 nm in diameter, which partially aggregate in larger structures observed in TEM images. After addition of TZV such large fibrils were no longer observed by any of the three techniques while smaller assemblies including rod-like fibrils just less than 10 nm in diameter appear to form instead. These fibrils could be identified as rod-like particles few tens of nanometres in length seen in TEM images, explaining the fact that they did not sediment during centrifugation.

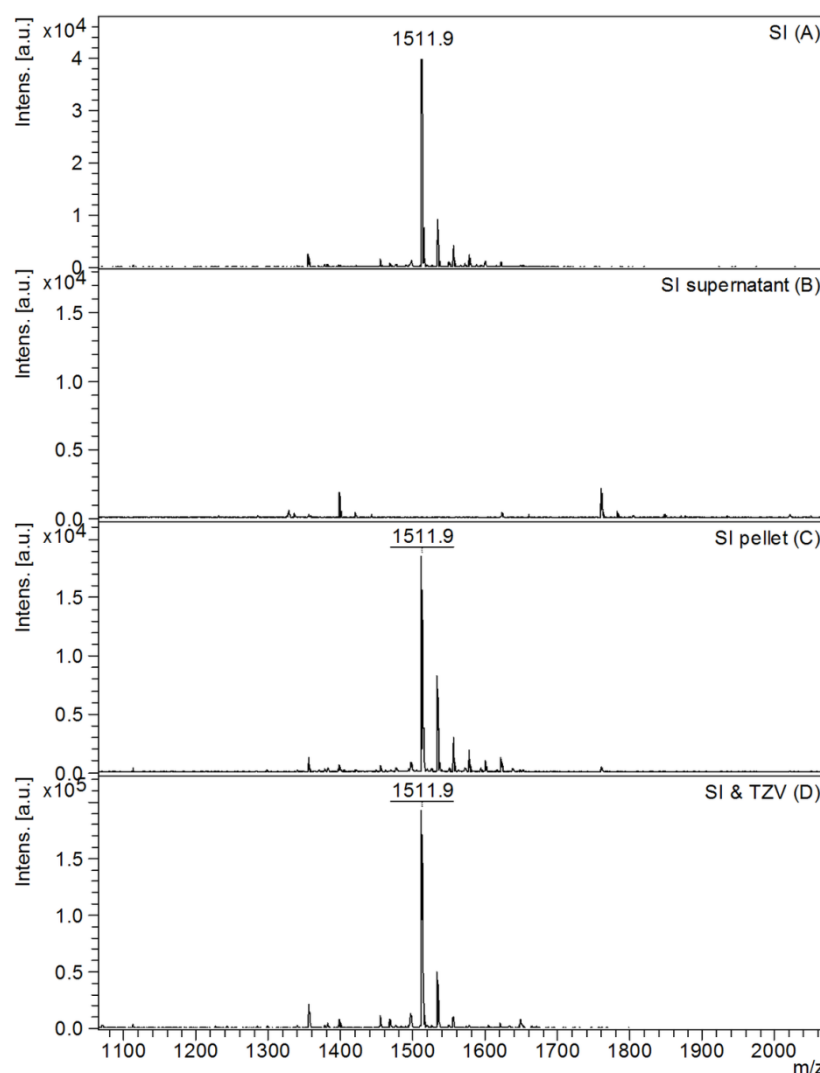


Figure 3. Mass-spectrometry (MS) analysis of (A) – SI fibrils solution; (B) SI fibrils supernatant MS analysis after 1000 g centrifugation for 5 min; (C) – SI fibrils pellet MS analysis after 1000 g centrifugation for 5 min; (D) – SI fibrils with triazavirine supernatant MS analysis after 1000 g centrifugation for 5 min.

The model of the peptide dimer was revealed by molecular dynamics simulations followed by trajectory analysis and molecular docking of the most frequent conformers [15]. There was an evident triazavirine interaction with arginine in dimer structure (Figure 4B). It is consistent with protein-nucleotide interaction data described in [6].

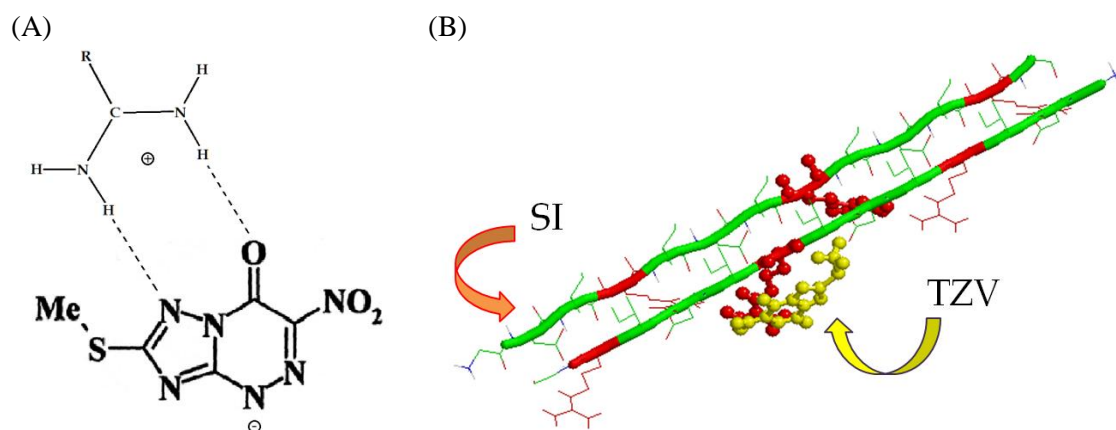


Figure 4. Hypothesis of triazavirine action on SI peptide fibrils. (A) Triazavirine can interact with arginine residue as described in [6] for nucleotide. (B) In dimer model the molecule of triazavirine (yellow) interacts with arginine residue side chain (red).

Currently substances liable to degradation of amyloid fibrils to components capable of participating in cellular catabolism are highly toxic. Therefore amyloidoses not currently be medicated. The presence of low-toxicity drugs with the potential to interact with the structural elements of amyloid fibrils holds great promise for the drug design for amyloidosis treatment. Thus, the data obtained in the study of interaction of triazavirine with iSCM-containing peptide may shed light on potential treatment of several conformational diseases.

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5. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

- [1] Sipe J D *et al* 2014 Nomenclature 2014: Amyloid fibril proteins and clinical classification of the amyloidosis *Amyloid* **21** 221–4
- [2] Karpenko I *et al* 2010 Antiviral properties, metabolism, and pharmacokinetics of a novel azolo-1,2,4-triazine-derived inhibitor of influenza A and B virus replication *Antimicrob. Agents Chemother.* **54** 2017–22
- [3] Farnsworth P N and Singh K 2000 Self-complementary motifs (SCM) in alpha-crystallin small heat shock proteins *FEBS Lett.* **482** 175–9
- [4] D'Auria G *et al* 2009 Self-assembling properties of ionic-complementary peptides *J. Pept. Sci.* **15** 210–9
- [5] Kabiri M, Bushnak I, McDermot M T and Unsworth L D 2013 Toward a mechanistic understanding of ionic self-complementary peptide self-assembly: role of water molecules and ions *Biomacromolecules* **14** 3943–50
- [6] Lancelot G, Mayer R and Hélène C 1979 Models of interaction between nucleic acids and

- proteins. Hydrogen bonding of arginine with nucleic acid bases, phosphate groups and carboxylic acids *Biochim. Biophys. Acta* **564** 181–90
- [7] Martellini J A *et al* 2009 Cationic polypeptides contribute to the anti-HIV-1 activity of human seminal plasma *FASEB J.* **23** 3609–18
- [8] Lump E *et al* 2015 A molecular tweezer antagonizes seminal amyloids and HIV infection *Elife* **4** e05397
- [9] Egorov V V *et al* 2013 Structural features of the peptide homologous to 6-25 fragment of influenza A PB1 protein *Int. J. Pept.* **2013** 370832
- [10] Kuklin A I, Islamov A K and Gordeliy V I 2005 Scientific Reviews: Two-Detector System for Small-Angle Neutron Scattering Instrument *Neutron News* **16** 16–18
- [11] Kuklin A I *et al* 2011 New opportunities provided by modernized small-angle neutron scattering two-detector system instrument (YuMO) *J. Phys. Conf. Ser.* **291** 12013
- [12] Kirilov A S *et al* 2004 Evolution of the SONIX Software Package for the YuMO Spectrometer at the IBR-2 Reactor *Instruments Exp. Tech.* **47** 334–345
- [13] Soloviev A G, Stadnik A V, Kuklin A I, Islamov A H and Solovieva T M 2003 SAS. The Package for Small-Angle Neutron Scattering Data Treatment. Version 2.4. Long Write-Up and User's Guide 22
- [14] Egorov V V *et al* 2008 Self-assembling capable peptides for development of bottom-up structured materials *Innovations* **6** 84–87
- [15] Altman M, Lee P, Rich A and Zhang S 2000 Conformational behavior of ionic self-complementary peptides *Protein Sci.* **9** 1095–105