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Structural characterization and biological properties of the amyloidogenic elastin-like peptide $(VGGVG)_3$

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ABSTRACT

The peculiar and unique properties of elastin are due to the abundance of hydrophobic residues and of repetitive sequences as XGGZG (X, Z = V, L or A). Unexpectedly, these sequences not only provide elasticity to the whole protein, but are also able to form amyloid-like fibrils. Even though amyloid fibrils have been associated for a long time to the development of serious disorders as Alzheimer's disease, recent evidence suggests that toxicity may be related to oligomeric species or to pre-fibrillar intermediates, rather than to mature fibrils. In addition, a number of studies highlighted the potential of "bio-inspired" materials based on amyloid-like nanostructures. The present study has been undertaken with the aim to characterize a chemically synthesized elastin-like peptide $(VGGVG)_3$. Structural and biological features were compared with those of peptides as poly(VGGVG) and VGGVG that, having the same amino acid sequence, but different length and supramolecular structure have been previously investigated for their amyloidogenic properties. Results demonstrate that a minimum sequence of 15 amino acids is sufficient to aggregate into short amyloid-like fibrils, whose formation is however strictly dependent on the specific VGGVG repeated sequence. Moreover, in the attempt to elucidate the relationship among aggregation properties, fibers morphology and biocompatibility, 3T3 fibroblasts were grown in the presence of VGGVG-containing elastin-like peptides (ELPs) and analyzed for their ability to proliferate, attach and spread on ELPs-coated surfaces. Data clearly show that amyloid-like fibrils made of $(VGGVG)_3$ are not cytotoxic at least up to the concentration of 100 μg/ml, even after several days of culture, and are a good support for cell attachment and spreading.

1. Introduction

In the last decades, much evidence has been provided on the interesting and pleiotropic characteristics of elastin and elastin-like polypeptides. By dissecting the elastin protein, it was demonstrated that i) the entire molecule, as well as sequences of reduced size and complexity, is able to self-assemble and to reveal elastic properties (Tamburro et al., 2003; Quaglino et al., 2009); ii) mechanical and biological properties can be tuned by selecting appropriate sequences (Trabbic-Carlson et al., 2003); iii) elastin-derived peptides, depending on their sequence, are able to self-assemble into classical elastin-like (Bellingham et al., 2003) or amyloid-like (Flamia et al., 2004; Tamburro et al., 2005) structures. Due to these peculiar features, elastin and elastin-like peptides are promising bio-nanomaterials with "smart" behavior (Rodriguez-Cabello, 2004; Channon and MacPhee, 2008) in terms of structural plasticity and mechanical stability.

Within this context, there are studies proposing that amyloid fibrils, due to their unique properties (i.e. easy production, low cost, outstanding mechanical stability and remarkably regular architecture) can be used as bio-inspired nanomaterials (Lashuel et al., 2000; MacPhee and Dobson, 2000; Cherny and Gazit, 2008; Mankar et al., 2011) suitable for developing nanowires for the electronics industry as well as biosensors and functionalized supports favoring cell attachment or differentiation (Yoshiike et al., 2007; Cherny and Gazit, 2008; Gras et al., 2008; Ohga et al., 2009; Cinar et al., 2012).

In support of the proposed biocompatibility of amyloid-like fibrils are the observations indicating that the primary cause of cytotoxicity is represented by pre-fibrillar aggregates, even from non-pathogenic proteins (Bucciantini et al., 2002, 2004), rather than the initial monomers and/or the insoluble fibrils. Even though mature fibril formation and aggregation into plaques were proposed to represent a protective mechanism to avoid the high intrinsic toxicity of oligomers or of pre-fibrillar intermediates (Bucciantini et al., 2002, 2004; Caughey and Lansbury, 2003; Sörgjerd et al., 2008), nevertheless, examples of toxicity

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associated with fibrils are present (Novitskaya et al., 2006; Gharibyan et al., 2007; Pieri et al., 2012). Therefore several issues concerning the toxicity of amyloidogenic molecules still remain unanswered.

Previous studies have shown that it is possible to synthesize elastin-like amyloidogenic peptides that share the presence of the XGGZG (where G is glycine whereas X, Z can be V = valine, A = alanine, L = leucine) motif along the sequence (Tamburro et al., 2005, 2010). Consistently, it has been demonstrated that the poly(VGGLG) and poly(VGGVG) synthetic polymers obtained by the poly-condensation of the XGGZG monomer (Flamia et al., 2004; Del Mercato et al., 2008) exhibit an amyloidogenic behavior.

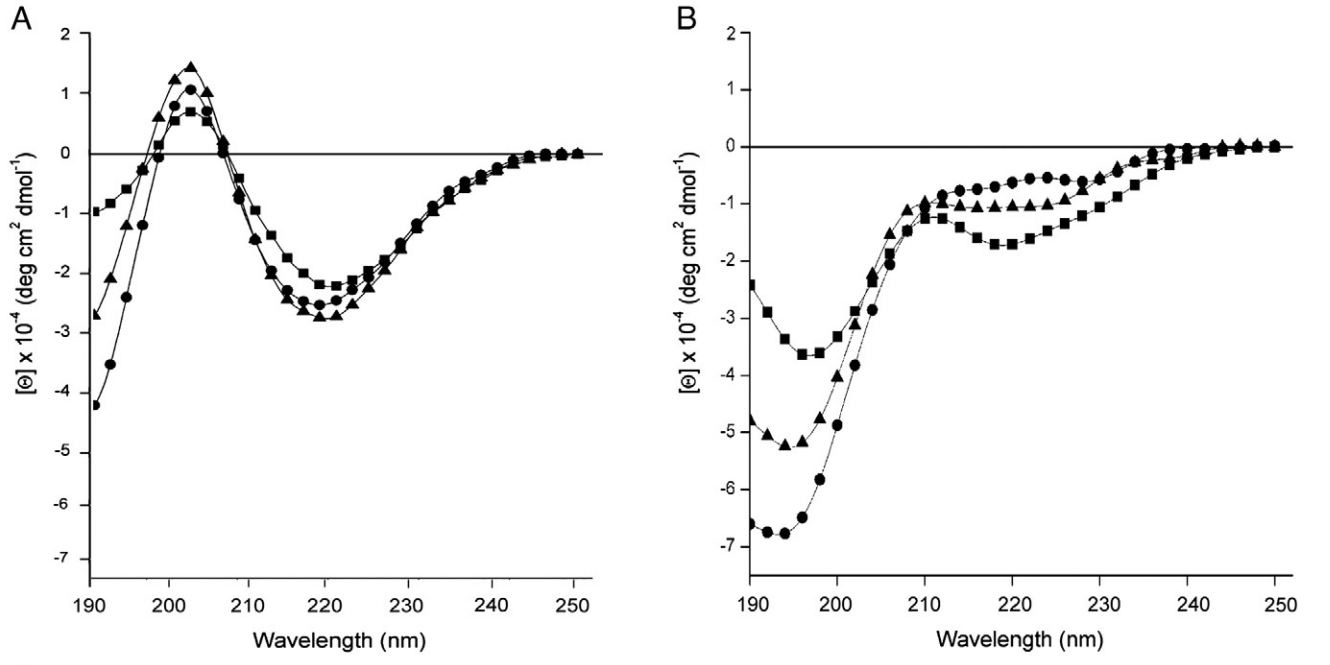
In the present study we have chemically synthesized and characterized a small peptide containing a 3-fold repeated motif VGGVG with the aim to confirm the hypothesis that: i) this short and specific sequence is necessary and sufficient to self-assemble into amyloid-like fibrils; ii) the population to which the fibrils belong is more homogeneous compared to those previously described; and iii) the $(VGGVG)_3$ peptide positively interacts with cells. Moreover, a further goal of the present study was to assess the toxicity of VGGVG polymers, if any, depending on the supramolecular organization of the peptide. Therefore, comparison has been also made among different VGGVG-containing elastin-like peptide (ELPs) as the newly synthesized $(VGGVG)_3$ and the amyloidogenic poly(VGGVG) polymer and the soluble pentapeptide VGGVG, which have been already characterized (Flamia et al., 2005).

The $(VGGVG)_3$ peptide was structurally characterized at molecular level by circular dichroism (CD), nuclear magnetic resonance (NMR), and Fourier transform infrared (FTIR) spectroscopy and, at supramolecular level, by atomic force microscopy (AFM), transmission electron microscopy (TEM) as well as thioflavin-T (ThT) and Congo red (CR) assays (Nilsson, 2004). Furthermore, cell viability including intracellular ROS content, as well as cell attachment and spreading on ELPs-coated surfaces were evaluated on mouse 3T3 fibroblasts. Additionally, attachment/spreading assays, CD and TEM were carried out on the scrambled peptide VVVGGGGVGVGVGGG (VGs) as control peptide in order to distinguish general effects of hydrophobic peptides versus the specific sequences that have been tested.

2. Results

2.1. CD spectroscopy

Fig. 1A shows CD spectra of the $(VGGVG)_3$ peptide recorded in aqueous solution at different temperatures. At 0 °C a negative and a positive band were centered at 218 and 200 nm, respectively, indicating a distorted beta-sheet together with unordered conformations (Manning et al., 1988). The increase of the temperature to 25 and 70 °C induces a slight decrease of the two bands due to the destabilization of beta-sheet conformation. Fig. 1B shows the CD spectra in aqueous solution of the VGs peptide. At 0 °C a strong negative band at 195 nm is visible together with a shoulder at 218 nm. These spectral findings are indicative of the presence of PPII conformation even if the expected positive band at 215 nm is absent. On increasing the temperature to 25 and 70 °C the reduction and the red shift of the negative band is observed together with the appearance of a small negative band centered at 218 nm. This finding is indicative of a conformational transition toward more folded conformations such as beta-turns. The temperature-induced folding of the peptide is probably due to hydrophobic interactions. For the evaluation of secondary structure content, analysis of CD spectra was performed with DICHROWEB analysis webserver (Whitmore and Wallace, 2008) using the CONTINLL



C

Temperature	Helix	Strand	Turn	Unordered
0°C	5	37	23	35
25°C	3	39	27	31
70°C	3	43	21	33

D

Temperature	Helix	Strand	Turn	Unordered
0°C	5	30	17	48
25°C	5	31	17	47
70°C	5	32	18	46

Fig. 1. Secondary structure analysis by circular dichroism (CD) spectroscopy. CD spectra of the $(VGGVG)_3$ peptide (A) and the VGs scrambled peptide (B) have been acquired in aqueous solution at temperature of 0 °C (circle), 25 °C (triangle), 70 °C (square). Tables show results of the deconvolution algorithm CONTINLL for secondary structure calculations applied to CD spectra of $(VGGVG)_3$ (C) and VGs peptides (D).

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