Available online at www.sciencedirect.com
ScienceDirect
The International Journal of Biochemistry & Cell Biology 39 (2007) 966–977
IJBCB
www.elsevier.com/locate/biocel

Destabilization of non-pathological variants of ataxin-3 by metal ions results in aggregation/fibrillogenesis

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Received 9 October 2006; received in revised form 10 January 2007
Available online 20 January 2007

Abstract

Ataxin-3 (AT3), a protein that causes spinocerebellar ataxia type 3, has a C-terminus containing a polyglutamine stretch, the length of which can be expanded in its pathological variants. Here, we report on the role of Cu^{2+}, Mn^{2+}, Zn^{2+} and Al^{3+} in the induction of defective protein structures and subsequent aggregation/fibrillogenesis of three different non-pathological forms of AT3, *i.e.* murine (Q6), human non-expanded (Q26) and human moderately expanded (Q36). AT3 variants showed an intrinsic propensity to misfolding/aggregation; on the other hand, Zn^{2+} and Al^{3+} strongly stimulated the amplitude and kinetics of these conformational conversions. While both metal ions induced a time-dependent aggregation into amyloid-like fibrillar forms, only small oligomers and/or short protofibrillar species were detected for AT3s alone. The rate and extent of the metal-induced aggregation/fibrillogenesis processes increased with the size of the polyglutamine stretch. Mn^{2+} and Cu^{2+} had no effect on (Q6) or actually prevented (Q26 and Q36) the AT3 structural transitions. The observation that Zn^{2+} and Al^{3+} promote AT3 fibrillogenesis is consistent with similar results found for other amyloidogenic molecules, such as β-amyloid and prion proteins. Plausibly, these metal ions are a major common factor/cofactor in the etiopathogenesis of neurodegenerative diseases. Studies of liposomes as membrane models showed dramatic changes in the structural properties of the lipid bilayer in the presence of AT3, which were enhanced after supplementing the protein with Zn^{2+} and Al^{3+}. This suggests that cell membranes could be a potential primary target in the ataxin-3 pathogenesis and metals could be a biological factor capable of modulating their interaction with AT3.

Keywords: Ataxin-3; Amyloid; Metal ions; Fibrillogenesis; Neurodegenerative disease

1. Introduction

Several neurodegenerative diseases are associated with protein misfolding and aggregation, with formation of insoluble proteinaceous deposits in different regions of the brain. Such aggregates may be both extra- and intracellular. Intracellular deposits may be found in both nuclei and cytoplasm, depending on the protein involved and the relative disease (Dobson, 1999; Ross & Poirier, 2004; Soto, 2003). The hallmark of these pathologies is the typical aggregation state of the proteins involved, which appear as amyloid structures, characterized by fibrillar appearance under electron microscopy, Congo red staining and birefringence, and cross-β-structure (Jin et al., 2003; Klunk, Jacob, & Mason, 1999; Sipe & Cohen,

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doi:10.1016/j.biocel.2007.01.012

2000). Based on these common traits, it is believed that such "protein conformational disorders", at least to some extent, share common mechanisms at a molecular level, which also are paralleled by similar clinical and histological features, particularly ataxia, cognitive and memory impairment, late onset and neuronal loss (Martin, 1999).

The most prominent diseases in this diverse grouping include Alzheimer (AD), Parkinson, prion diseases, Huntington and related polyglutamine (polyQ) disorders, including several forms of spinocerebellar ataxias (Dobson, 1999; Fischbeck, 2001; Soto, 2003; Stefani & Dobson, 2003).

Many amyloid diseases are inherited: this is the case, for instance, of familial forms of AD (Selkoe, 2001) and polyQ diseases (Orr, 2001). Others are transmissible, as in the case of some prion diseases (Prusiner, 1998). Furthermore, it is well reported that environmental agents may also favor the onset of amyloid diseases (Alexandrescu, 2006; Plassman & Breitner, 1996; Spires & Hannan, 2005). In particular, it has been suggested that the accumulation of metal ions in the brain plays a role in their pathogenesis (Bush, 2000; Bush, Masters, & Tanzi, 2003; Gaeta & Hider, 2005; Maynard, Bush, Masters, Cappai, & Li, 2005; Sayre, Perry, Atwood, & Smith, 2000). Accordingly, binding of metals to many neurodegenerative disease-related proteins has been found to affect the pathways of abnormal folding and lead to the generation of amyloid fibrils *in vitro* (Bocharova, Breydo, Salnikov, & Baskakov, 2005; Bush, 2000; Ricchelli et al., 2006; Ricchelli, Drago, Filippi, Tognon, & Zatta, 2005; Uversky, Li, & Fink, 2001).

Here, we report investigations into the effect of different metals on the structure and the stability of ataxin-3 (AT3), a 42-kDa, widely expressed protein, which causes Machado–Joseph disease/spinocerebellar ataxia type 3. Neurodegeneration occurs via selective damage, mainly of cerebellar dentate neurons, basal ganglia, brain stem and spinal cord (Donaldson et al., 2003; Perez, Paulson, & Pittman, 1999). Its primary structure encompasses a conserved N-terminal region, the so-called Josephin domain, and a less conserved flexible C-terminus containing a polyQ stretch (Albrecht, Golatta, Wullner, & Lengauer, 2004; Chow, Mackay, Whisstock, Scanlon, & Bottomley, 2004; Masino et al., 2003). In its normal form, it contains 12–36 glutamines, whereas the length of the polyQ in its pathological variants lies in the range 55–84. Glutamine expansions beyond the critical size are thought to induce conformational modifications in the host protein and promote protein aggregation by acting as "polar zippers" (Perutz, 1996, 1999). This results in the formation of insoluble, largely nuclear aggregates (Paulson, 1999), although the physiological localization of the protein is cytoplasmic (Paulson, Das, et al., 1997). Intriguingly, both expanded and non-expanded forms of AT3 are observed within nuclear inclusions.

Different physiological roles have been proposed for AT3, including possible involvement in transcriptional control (Li, Macfarlan, Pittman, & Chakravarti, 2002) and regulation of protein degradation. In reference to the latter function, recent papers showed that the highly conserved N-terminal Josephin domain of AT3 binds ubiquitin and has ubiquitin hydrolase activity (Scheel, Tomiuk, & Hofmann, 2003). This was confirmed in a recent paper that reported the solution structure of the Josephin domain, determined by NMR spectroscopy (Nicastro et al., 2005).

In our previous studies (Marchal et al., 2003; Shehi et al., 2003) we investigated three different forms of AT3, *i.e.* murine (Q6), human non-expanded (Q26) and human moderately expanded (Q36). Q6 and Q26 AT3 are amazingly heat-stable, at least as far as the secondary structure is concerned, however our studies showed that the Q36 variant underwent an alpha–beta transition starting at about 40 °C, followed by amyloid fibrils formation. This made it possible to modulate the rate of amyloidogenesis and assess the effects of potentially antiamylodogenic compounds.

To the best of our knowledge, to date no studies have examined the effect of metal ions on stability and aggregation of proteins carrying either normal or pathological polyQ stretches. This prompted us to assay the ability of a group of metal ions, namely Al^{3+}, Zn^{2+}, Cu^{2+} and Mn^{2+}, to affect the stability and the structure of three aforementioned variants of ataxin-3. We compared the effects of these metal ions on AT3 with those previously observed on other neurodegenerative disorder-related proteins, such as β-amyloids and prion proteins (Ricchelli et al., 2005, 2006). In order to identify which domains of AT3 preferentially interact with metal ions, we also performed selected experiments on the 1–182 fragment lacking the polyQ tract (Josephin domain).

By studying the effects of a number of metals on AT3s carrying differently expanded polyQs, we aimed to assess: (i) whether metal ions favor misfolding, aggregation and fibrillogenesis; (ii) whether and how the expansions may affect the process. We found that Zn^{2+} and Al^{3+} are the only metals among those assayed that enhanced protein aggregation, and that their effect increases with an increase in size of the polyQ expansion.