Coordination Chemistry Reviews ■■ (2016) ■■–■■

Contents lists available at ScienceDirect

Coordination Chemistry Reviews

journal homepage: www.elsevier.com/locate/ccr

Review

Challenges in studying the structures of metal-amyloid oligomers related to type 2 diabetes, Parkinson's disease, and Alzheimer's disease

Vered Wineman-Fisher [a,b], Daniel Nir Bloch [a,b], Yifat Miller [a,b,*]

[a] Department of Chemistry, Ben-Gurion University of the Negev, P.O. Box 653, Beersheba 84105, Israel
[b] Ilse Katz Institute for Nanoscale Science and Technology, Ben-Gurion University of the Negev, Beersheba 84105, Israel

Contents

ARTICLE INFO

Article history:
Received 28 March 2016
Accepted 17 April 2016
Available online

Keywords:
Alzheimer's disease
Amyloid mechanism
Amyloid structural oligomer
Metal
Parkinson's disease
Type 2 diabetes

ABSTRACT

Amylin, amyloid beta (Aβ), and α-synuclein are peptides and proteins that belong to a large family of amyloids, which are involved in the progress of amyloidogenic diseases: amylin in type 2 diabetes, Aβ in Alzheimer's disease, and α-synuclein in Parkinson's disease. Amyloids self-assemble to form aggregates such as oligomers and fibrils. It is known that the amyloid oligomers are the toxic species in these diseases. It is also well documented that metal ions interact with these amyloids to enhance the formation of amyloid oligomers. However, the mechanisms that allow metal ions to interact with these amyloid oligomers are elusive. Thus, to obtain insights into these mechanisms, it is necessary to determine the atomic structures of metal-full-length amyloid oligomer complexes. There many challenges when using current conventional experimental tools to observe the structures of metal-full-length amyloid oligomer complexes at atomic resolution. However, current and future computational studies may help to elucidate the various conformations of metal-full-length amyloid oligomer complexes. This review describes the challenges that must be addressed in experimental and computational studies to obtain a more complete understanding of the mechanisms that allow metal ions bind to these amyloid oligomers.

1. Introduction

Amyloidogenic diseases such as Type 2 diabetes (T2D), Parkinson's disease (PD), and Alzheimer's disease (AD) comprise major global health problems. In these amyloidogenic diseases, the amyloids (which are peptides and proteins) self-assemble into aggregates, such as oligomers and fibrils: amyloid beta (Aβ) in AD, amylin in T2D, and α-synuclein in PD. Metals such as zinc, iron, and copper are essential for most important metabolic processes [1], and they are also involved in many crucial functions in the nervous system, e.g., iron supports the brain's high respiratory rate and the synthesis of neurotransmitters [2,3]. However, interruption of the delicate balance of metals has a critical role in amyloid aggregation. Metals are important in metabolic process but poisonous at moderate or high concentrations, so it is not surprising that growing numbers of amyloidogenic diseases, particularly neurodegenerative diseases, such as PD and AD, are linked to metal ion homeostasis.

Many reviews have described the roles of metal ions in binding to amyloids and the subsequent promotion of their aggregation [4–7]. However, clinical and *in vivo* studies cannot provide information about how metal ions bind to amyloids and the mechanisms involved. Furthermore, it is extreme challenging for *in vitro* and *in silico*

* Corresponding author. Tel.: +972 86428705; fax: +972 86428709.
E-mail address: ymiller@bgu.ac.il (Y. Miller).

http://dx.doi.org/10.1016/j.ccr.2016.04.010
0010-8545/

studies to identify the specific metal binding sites in amyloids, particularly to the full-length amyloid oligomers (which are known to be the toxic species) at atomic resolution. Therefore, it is important to investigate the interactions of metal ions with the full-length of amyloid oligomers because it is necessary to elucidate the effects of metal ions on the folded amyloid oligomers. However, investigations of the binding of metal ions to fragments of amyloids (monomers or oligomers) fail to provide important knowledge regarding the structural effects of these metal ions on all of the domains of amyloids. Therefore, characterizing the structural properties of full-length amyloid oligomers complexed with metal ions and their relative stabilities is important for understanding the effects of metal ions on aggregation. The following key issues have emerged in the last decade: (1) where and how metal ions bind amyloids; (2) the specific aspects of the coordination chemistry of metal ions that define the chemical reactivity and that influence the three-dimensional structure of amyloids; (3) whether different conformations with different residues coordinate the metal ions and their identities; and (4) the preferred conformations.

Previously, a review by Miller et al. [8] in 2012 focused on metal binding to Aβ, but they did not discuss the challenges that affect the structural characterization of metal-Aβ oligomers and other metal amyloid oligomers. Since the review by Miller et al., there has been little progress in the structural characterization of metal-amyloid oligomers, such as metal-amylin or metal-α-synuclein oligomers. Therefore, it is important to facilitate future research into the structural characterization of metals that bind amylin oligomers and α-synuclein oligomers.

The present review focuses on the challenges that affect the investigation of specific metal binding sites in amyloids. Various experimental methods are described for investigating the metal binding sites and proposals are made for how *in silico* studies might tackle these challenges. The metal binding sites in Aβ, amylin, and α-synuclein are discussed based on examples from several studies that have attempted the structural characterization of metal-Aβ oligomers, specifically the challenges that must be addressed when investigating these oligomers and other metal-amyloid oligomers. Finally, the different metal binding sites for various metal ions in these amyloids are summarized. In addition, the challenges of *in vitro* and *in silico* studies are described to provide insights into the polymorphic states of metal-amyloid oligomers based on the full-length amyloids at atomic resolution. Understanding the mechanisms and the range of structural features in metal-full-length amyloid aggregates is crucially important for effective drug design to reduce aggregate formation.

2. Structural characterization of metal-amyloid oligomers

2.1. Structural characterization of metal-aβ oligomers

Among all of the amyloids, the metal-binding sites in Aβ have been studied most widely. Thus, the Aβ binding sites for Zn^{2+} and Cu^{2+} have been studied extensively by *in vitro* studies, but there is a lack of data for Fe^{3+}. Therefore, the present review focuses only on the Zn^{2+} and Cu^{2+} binding sites in Aβ. However, the metal-binding sites that have been proposed based on *in vitro* studies were determined only for Aβ monomers and not for Aβ oligomers. Furthermore, all of the *in vitro* studies focused only on Aβ peptide fragments and not the full-length Aβ oligomer. Previously, Miller et al. reviewed all of the binding sites for Zn^{2+} and Cu^{2+} in Aβ monomers according to experimental *in vitro* studies [8]. Obviously, the binding sites are pH dependent [8].

The possible residues that coordinate with Cu^{2+} in fragments of Aβ monomers according to experimental studies are illustrated in Fig. 1 and they were reviewed by Miller et al. [8]. The possible Cu^{2+} coordinating residues in Aβ monomers based on experimental

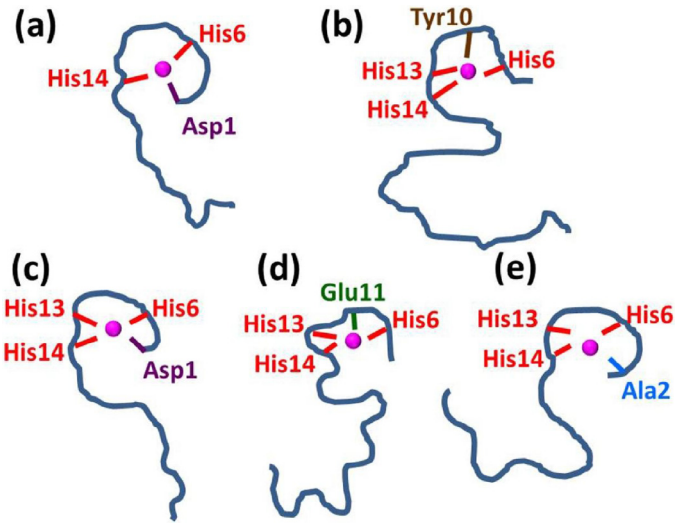

Fig. 1. Different models based on experimental data for the Cu^{2+} binding sites in Aβ monomers. Rat Aβ: (a) Ref. [9]; and human Aβ: (b) Ref. [10–16], (c) Ref. [17], (d) Ref. [18], (e) Ref. [19].

studies include histidine residues (His6, His13, and His14), tyrosine (Try10), aspartate or glutamate (Asp1, Glu3, Asp7, Glu11, Glu22, and Asp23), methionine (Met35), deprotonated amides in the peptide backbone, or carbonyl groups [10,20–24]. The most common residues are located at the N-terminus of Aβ. The binding site for Cu^{2+} may comprise three histidine residues (His6, His13, and His14) [10–16] and Tyr10 [16]. Studies have also demonstrated that the Cu^{2+} binding site may involve Asp1 [17,18] or Glu11 [18]. A recent study of the Cu^{2+} binding site in the rat $Aβ_{1–28}$ fragment showed that Cu^{2+} binds to Asp1, His6, and His14 [9]. His13 is absent from rat $Aβ_{1–28}$ [17]. Recent simulations have suggested that Cu^{2+} coordinates with His13 and His14 in each of two adjacent Aβ peptides [25]. Drew et al. [19] suggested that Ala2-carbonyl is an oxygen ligand of Cu^{2+} in Aβ peptides. These studies have proposed various possible conformers. The *in vitro* studies indicate that Cu^{2+} binds to the N-terminal of Aβ monomers. The interactions between Cu^{2+} and fragments of $Aβ_{1–16}$ monomers have been studied computationally by Sodupe's group using density functional theory [26–28]. However, a combination of experimental and computational studies of the interactions between Cu^{2+} and Aβ fibrils indicated that Cu^{2+} can also bind to the C-terminal of Aβ [29], although the binding of Cu^{2+} ions to the full-length Aβ oligomers at atomic resolution was solved in computational study by considering a Cu^{2+}:amyloid ratio of only 1:1 [29]. In general, the conformations of Aβ oligomers detected experimentally based on Cu^{2+} binding sites in Aβ monomers appear to depend on populations with various morphologies under different conditions.

The coordination of Cu^{2+} (an open shell (3d9) metal cation) with Aβ monomers has been investigated using electronic structure methods [30]. The binding of dioxygen to the Cu^{+}–$Aβ_{1–16}$ monomer [28] and of Fe^{2+}/Fe^{3+} with only His13 and His14 in the Aβ monomer [31] have been studied, where the electronic structure methods are more accurate than molecular mechanics methods. However, solvated Cu^{+}–Aβ oligomers are the toxic species, and thus it is important to study these species. Solvated metal-Aβ oligomers have not been studied using electronic structure methods. Currently, the electronic structure methods have limitations when studying solvated metal-Aβ oligomers, where the relatively large number of atoms does not permit computations within a reasonable timescale for these species. Thus, molecular mechanics methods are more suitable for these relatively large biological systems. To address such limitations, future quantum mechanics/molecular

Please cite this article in press as: Vered Wineman-Fisher, Daniel Nir Bloch, Yifat Miller, Challenges in studying the structures of metal-amyloid oligomers related to type 2 diabetes, Parkinson's disease, and Alzheimer's disease, Coordination Chemistry Reviews (2016), doi: 10.1016/j.ccr.2016.04.010