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Metals accelerate the formation and direct the structure of amyloid fibrils of NAC

Ayesha Khan [a], Alison E. Ashcroft [b], Valerie Higenell [a], Olga V. Korchazhkina [c], Christopher Exley [a,*]

[a] *Birchall Centre for Inorganic Chemistry and Materials Science, Lennard-Jones Laboratories, Keele University, Staffordshire ST5 5BG, UK*
[b] *School of Biochemistry and Molecular Biology, University of Leeds, Leeds LS2 9JT, UK*
[c] *Institute for Science and Technology in Medicine, Keele University, Staffordshire ST4 7QB, UK*

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Abstract

Non-beta amyloid component of Alzheimer's disease amyloid or NAC is a highly amyloidogenic peptide consisting of 35 amino acids which was first identified associated with senile plaques in the Alzheimer's disease brain. It is a fragment of the presynaptic protein α-synuclein and, as such, it is implicated in the aetiologies of both Alzheimer's (AD) and Parkinson's (PD) disease. Metals are involved in the aggregation of amyloidogenic peptides such as beta amyloid (Aβ), British amyloid peptide (ABri) and α-synuclein though nothing is yet known about how they might influence the aggregation of NAC. We show herein that NAC will form β-pleated conformers at a peptide concentration of only 2.0 μM and that metals, and Zn(II) and Cu(II) in particular, accelerate the formation of these fibrils. Cu(II) and Zn(II) did not influence the diameter or general structure of the fibrils which were formed though many more shorter fibrils were observed in their presence and these shorter fibrils were highly thioflavin T positive and they were efficient catalysts of the redox cycling of added Fe(II). By way of contrast, β-pleated conformers of NAC which were formed in the presence of Al(III) showed much lower levels of thioflavin T fluorescence and were poorer catalysts of the redox cycling of added Fe(II) and these properties were commensurate with an increased abundance of a novel amyloid morphology which consisted of twisted fibrils with a periodicity of about 100 nm. These spirals of twisted fibrils were especially abundant in the presence of added Al(III) and it is speculated that NAC binding of Al(III) may be important in their formation and subsequent stability.
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1. Introduction

The non-Aβ component of AD amyloid (NAC) was first identified co-localised with Aβ in senile plaques [1]. It is an hydrophobic peptide of 35 amino acids in length and is a fragment of the presynaptic protein α-synuclein. It is not known how it is formed from α-synuclein or if it has a function in vivo. It has been shown to bind Aβ [2], to be bound by its precursor protein α-synuclein [2] and to exhibit isoform specific binding of apolipoprotein E [3]. It is amyloidogenic [4] and will seed the formation of Aβ fibrils which, in turn will seed the formation of NAC fibrils [5]. The latter are neurotoxic [6] and are believed to induce apoptosis in neurones via the formation of reactive oxygen species (ROS) and inflammatory markers such as NF-κB [7]. The former almost certainly involving the catalysis of iron-driven Fenton chemistry [8].

* Corresponding author. Fax: +44 1782 712378.
E-mail address: c.exley@chem.keele.ac.uk (C. Exley).

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There are no reports of the influence of metals on NAC amyloidosis. Metals, and in particular, Al(III), Fe(III), Cu(II) and Zn(II) accelerate the aggregation of α-synuclein [9] and trigger fibril formation of non-fibril forming methionine-oxidised α-synuclein [10]. In PD metals are co-precipitated with amyloid fibrils of α-synuclein in Lewy bodies in dopaminergic neurones of the substantia nigra [11,12]. The NAC region is absolutely necessary for α-synuclein to form fibrils [13] and it would be interesting to speculate that it is this region which is also involved in α-synuclein binding of metals. Herein we have investigated the influence of Al(III), Fe(III), Cu(II) and Zn(II) on NAC amyloidosis and we have demonstrated effects upon the rate of formation of fibrils and the morphology of fibrils formed during long term incubation.

2. Materials and methods

NAC was purchased from Bachem (Saffron Walden, UK) and 1 mg/mL stock solutions (ca. 0.27 mM NAC for a peptide content of 88%) were prepared according to manufacturers instructions. This concentration of NAC was not soluble in ultrapure water. It was soluble in a 1/40 mixture of formic acid and ultrapure water. Stock solutions were centrifuged prior to use to reduce the possibility of any NAC amyloid seeds remaining in solution. NAC peptide stock was then diluted into Krebs-Henseleit (KH) medium (NaCl, 123.5 mM; KCl, 4.8 mM; $MgSO_4$, 1.2 mM; $CaCl_2$, 1.4 mM; glucose, 11.0 mM) which was buffered at pH 7.40 ± 0.05 with 100 mM PIPES (1,4-piperazinediethanesulfonic acid) and also included either no added metal or 10 μM of Al(III), Fe(III), Cu(II) or Zn(II) to give metal–NAC preparations which included the peptide at a concentration of 2.0 μM. The NAC only preparations included <0.02 μM Cu(II)/Zn(II), 0.39 ± 0.01 μM Al(III) and 0.17 ± 0.01 μM Fe(III) as contaminant metals. The in vivo concentration of monomeric NAC is unknown. We have chosen to use a concentration which has not hitherto been shown to spontaneously form amyloid fibrils in vitro but which is above the nM concentrations of NAC which are known to bind apolipoprotein in vitro [3]. Each of the metals was added to buffered KH medium from certified standards of their nitrate salts (Perkin–Elmer, Beaconsfield, UK) and the metal-containing media were subsequently equilibrated for 24 h at 37 °C prior to the addition of the peptide. The latter improved the hydrolytic stability of metal equilibria which, particularly with respect to Al(III), can be very slow to equilibrate under physiological-like conditions [14]. The chosen concentration of total metal, 10 μM, is physiologically relevant for Fe(III), Cu(II) and Zn(II) and pathophysiologically significant for Al(III). Control solutions which did not include NAC but did include the requisite concentration of formic acid were also prepared for each of the NAC preparations. The buffered metal–NAC preparations were used in a short term and a long term experiment. In the latter 0.05% w/v sodium azide (NaN_3) was included in each preparation to prevent the growth of micro-organisms during the incubation of the preparations at 37 °C for up to 12 weeks. Sodium azide was not included in preparations used in the short term experiment as the period of incubation was only 24 h. All preparations were made up in PTFE vials and were only agitated prior to sampling.

The aggregation of NAC in the absence and presence of each of the metals was followed for either 24 h (short term experiment; samples taken at 1 and 24 h) or 12 weeks (long term experiment; samples taken at 1 h, 1, 2, 4, 8 and 12 weeks) using a combination of techniques including: (i) thioflavin T fluorescence (ThT) to identify the formation of β-pleated sheets of amyloid [15]; (ii) reverse phase high performance liquid chromatography (RP HPLC) to identify and separate NAC monomers [16]; (iii) electrospray mass spectrometry (ESMS) to confirm the identity of eluted NAC by determination of its molecular mass [17] and (iv) transmission electron microscopy (TEM) incorporating negative staining with 2% uranyl acetate to confirm visually the formation of amyloid fibrils and other forms of precipitated NAC [18]. Each of these techniques was used as described previously in the cited reference. In addition to the aggregation status of NAC we also determined the propensity for the peptide in each preparation to catalyse the redox cycling of Fe(II). We have shown that in physiological-like media in the absence of added peptide the auto-oxidation of 5.0 μM Fe(II) to Fe(III) is approximately complete within 30 min. In the presence of $Aβ_{42}$ the auto-oxidation of the same concentration of added Fe(II) appeared to be inhibited though the further observation that under the same conditions Fe(II) could be generated from the addition of Fe(III) suggested that rather than inhibit the auto-oxidation of Fe(II) the peptide accelerated the Fe(II)/Fe(III) redox cycle. These experiments are described in full elsewhere (Khan et al., submitted) and are reproduced for NAC herein. Briefly, as for $Aβ_{42}$, Fe(II) was added to a sample of each NAC preparation to give a final [Fe(II)] of 5.0 μM and the samples were incubated at 37 °C for 30 min to allow the auto-oxidation of Fe(II) to take place. After 30 min an excess of 1,10-phenanthroline was added and the [Fe(II)] determined spectrophotometrically as the Fe(II)-phenanthroline complex [19]. The influence of NAC on the auto-oxidation of the added Fe(II) was determined by comparing the concentration of Fe(II) measured in the presence of NAC with the concentration of Fe(II) in the equivalent peptide-free control.

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