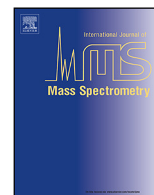
International Journal of Mass Spectrometry 391 (2015) 47–53

Contents lists available at ScienceDirect

International Journal of Mass Spectrometry

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



On the structure of the copper–amylin complex

Isabel Riba[a,b], Perdita E. Barran[c], Garth J.S. Cooper[a,b,d,e], Richard D. Unwin[a,b,*]

[a] Centre for Advanced Discovery and Experimental Therapeutics, Central Manchester University Hospitals NHS Foundation Trust (CMFT), Manchester M13 9WL, United Kingdom
[b] Centre for Endocrinology & Diabetes, Institute of Human Development, Manchester Academic Health Science Centre, University of Manchester, Manchester, United Kingdom
[c] Michael Barber Centre for Collaborative Mass Spectrometry, The School of Chemistry, Manchester Institute of Biotechnology, The University of Manchester, 131 Princess Street, Manchester M1 7DN, United Kingdom
[d] School of Biological Sciences, Faculty of Science, and Department of Medicine, Faculty of Medical and Health Sciences, University of Auckland, Auckland, New Zealand
[e] Department of Pharmacology, Medical Sciences Division, University of Oxford, Oxford OX1 3QT, United Kingdom

ARTICLE INFO

Article history:
Received 15 May 2015
Received in revised form 4 September 2015
Accepted 4 September 2015
Available online 16 September 2015

Keywords:
Amylin
Copper
Ion-mobility
Mass spectrometry

ABSTRACT

Amylin amyloid deposition is a common feature in the pancreas of type-2 diabetic (T2D) patients, and may play a role in islet beta-cell death and disease development. Type-2 diabetes is also characterised by a dysregulation of systemic copper. While the relationship between these features is unclear, previous work has shown that copper can form a complex with amylin, which in turn prevents amylin misfolding, oligomerisation and fibril formation, and that this in turn decreases amylin-mediated cytotoxicity. To date, the nature of this interaction has not been determined. In this study, we have used ion mobility mass spectrometry to probe the effects of copper on amylin oligomer formation and to define the binding site(s) of copper on the amylin peptide. Here we show that there are two distinct sites to which copper can bind. The first is at a location in the C-terminal 5 amino acids of the peptide. The second is on Ala25, a residue which is known to be critical for the misfolding ability of human amylin. This interaction is not observed under standard mass spectrometry (acidic) conditions but is present at physiological pH values. These data further characterise the amylin–copper interaction, and may suggest a potential trigger for amylin misfolding in T2D.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Amylin is a 37-amino acid peptide which is secreted by the pancreas together with insulin. It was first discovered as the key component of amyloid plaques in the islets of Langerhans in patients with diabetes [1]. These plaques are present in approximately 90% of patients with diabetes [2]. Research over recent years has shown that human amylin is an intrinsically disordered protein. In its monomeric state, the peptide is predominantly random coils, although some studies have suggested presence of alpha-helices at the N-terminus [3]. However, amylin readily forms structures containing beta-hairpins, and it is these structures which appear to be key to dimerization [4,5]. This innate ability to 'misfold' into beta-sheets, aggregate and form cytotoxic species [6–10] is likely to contribute to the development of human diabetes [11].

The sequence of amylin is critical in the formation of these aggregates. While amylin is highly conserved between species, it has been shown that rodent amylin does not aggregate readily, and this has been associated with changes in specific parts of the amylin sequence, notably around the sequence responsible for amylin misfolding at residues 20–29 (Fig. 1). Indeed, mutation of human amylin by replacing the amino acids at 25, 28 and 29 with proline forms the basis of the anti-diabetic medicine Pramlintide (Symlin), an active amylin analogue which does not misfold [12].

Another key feature of diabetes is that patients have elevated serum copper levels [13]. Given the knowledge that for other amyloidogenic peptides such as α-synuclein (Parkinson's disease) and amyloid β_{1-42} (Alzheimer's disease) copper can accelerate amyloid formation [14], a small number of studies have been performed to determine whether the same is true of copper binding to amylin [15–17].

Perhaps paradoxically, however, it appears that the reverse may be true, and that copper bound to amylin actually inhibits

* Corresponding author at: Centre for Advanced Discovery and Experimental Therapeutics, Central Manchester University Hospitals NHS Foundation Trust (CMFT), Manchester M13 9WL, United Kingdom. Tel.: +44 0161 701 0244.
E-mail address: r.unwin@manchester.ac.uk (R.D. Unwin).

http://dx.doi.org/10.1016/j.ijms.2015.09.001
1387-3806/© 2015 Elsevier B.V. All rights reserved.

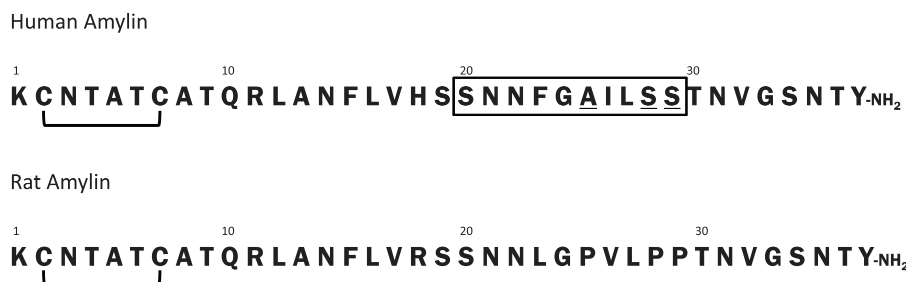


Fig. 1. Sequences of human and rat amylin. These peptide hormones are highly homologous, with identical sequence at the N-terminal half of the peptide, including a disulphide bond between Cys^2 and Cys^7 and an amide modification at the C-terminus. The region thought to be responsible for the misfolding of human amylin is highlighted in a box, with the residues which are mutated to Pro residues to produce an active but non-misfolding hormone (pramlintide) underlined.

amyloid formation. This raises a series of questions regarding the role of copper–amylin interaction, both in respect of normal physiological function and the onset of diabetes. It has been noted that while copper has been shown to decrease amylin fibril formation, it is the smaller oligomers which are beyond the reach of assays such as Thioflavin T (ThT) fluorescence and transmission electron microscopy [15] which are indeed more cytotoxic. More recent data from Lee et al. [18], however, have shown that the addition of copper can protect cells from amylin-mediated cell death. Circular dichroism studies suggest that copper addition blocks beta-sheet formation. Cellular assays in this study suggest that while both amylin only and copper only are cytotoxic (*via* formation of amyloid and reactive oxygen species (ROS) respectively), a combination reduces amyloid and ROS levels and protects from cell death. This is in direct contrast to data from Sinopoli et al. [17], which suggest that the addition of copper to amylin increases cytotoxicity, although these data were obtained using the amylin 17–29 fragment, and this effect was not as marked with the full length human sequence. It has also been shown that in addition to affecting amylin structure, the binding of Cu also inhibits amylin cleavage by insulin-degrading enzyme [19], particularly around the sites at Asn^{22}-Phe^{23} and Leu^{27}-Ser^{28}.

The role of these processes in disease development remains unclear. It would therefore be of use to accurately define the copper–amylin complex in more detail, including the site and nature of the copper interaction, to shed further light on these processes. One previous attempt at mapping the interactions between copper and amylin identified a gas phase interaction somewhere between Asn^{22} and Ile^{26}. In this study, we have used ion mobility mass spectrometry to define the nature of the interactions of copper with amylin.

2. Materials and methods

Amylin was obtained from Bachem (Switzerland, sequence shown in Fig. 1), dissolved in 500 μL of hexafluoroisopropanol, vortexed for 10 min and kept at room temperature overnight in the dark for deseeding. Then, it was aliquoted in 10 equal fractions (1 mg each), dried in a Savant RVT4104 refrigerated vapour trap (Thermo Scientific, Hemel Hempstead, UK) and kept in the freezer at −20 °C until further analysis.

Copper chloride, water, ammonium acetate, Thioflavin T and formic acid were all purchased from Sigma–Aldrich (Poole, UK).

2.1. Electrospray and ion mobility experiments

Electrospray mass spectrometric data were acquired using a Waters Synapt G2S (Waters Corporation, Manchester, UK) equipped with MassLynx 4.1. Samples were introduced using in-house prepared nanospray glass emitter tips with a platinum wire to allow ionisation, as described previously [20]. The capillary voltage, cone voltage and source temperature were all set according to manufacturer's guidelines, typically at 1.9 kV, 40 V and 60 °C respectively. The ion mobility wave was operated at 1300 m/s with a constant wave height of 40 V and data were acquired over 500 to 4000 *m*/*z* range. For fragmentation experiments, collision energy was set at 45 V.

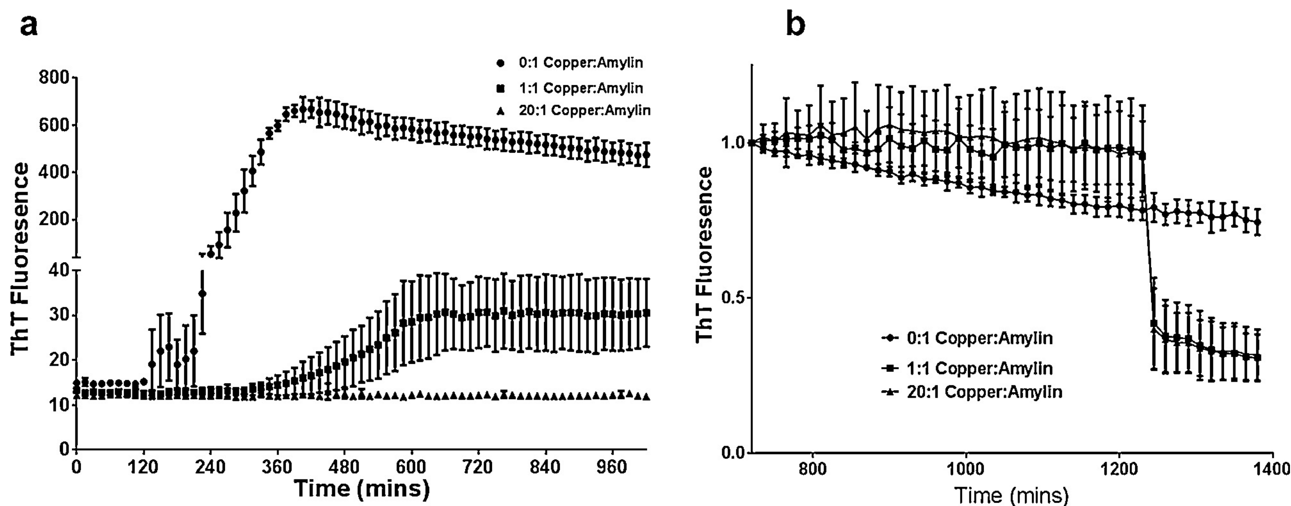


Fig. 2. Copper prevents formation of human amylin fibrils. a) ThT fluorescence assay of amylin in the presence or absence of copper, showing delayed inhibition of fibril formation with equimolar amounts of copper (as $CuCl_2$) and complete inhibition with copper in a 20× molar excess ($n = 3$, mean ± s.d.). b) The addition of copper to preformed steady state fibrils partially reverses misfolding, reducing the amount of beta-sheet present ($n = 3$, mean ± s.d.).

Download English Version:

https://daneshyari.com/en/article/7604273

Download Persian Version:

https://daneshyari.com/article/7604273

Daneshyari.com