Biophysical Chemistry 218 (2016) 58–70

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: http://www.elsevier.com/locate/biophyschem

Regulation of the assembly and amyloid aggregation of murine amylin by zinc

Luiza C.S. Erthal [a], Adriana F. Marques [a], Fábio C.L. Almeida [a], Gustavo L.M. Melo [a], Camila M. Carvalho [a], Leonardo C. Palmieri [a], Katia M.S. Cabral [a], Giselle N. Fontes [b], Luís Maurício T.R. Lima [a,b,c,*]

[a] School of Pharmacy, Federal University of Rio de Janeiro – UFRJ, CCS, Bss24, Ilha do Fundão, 21941-590 Rio de Janeiro, Brazil

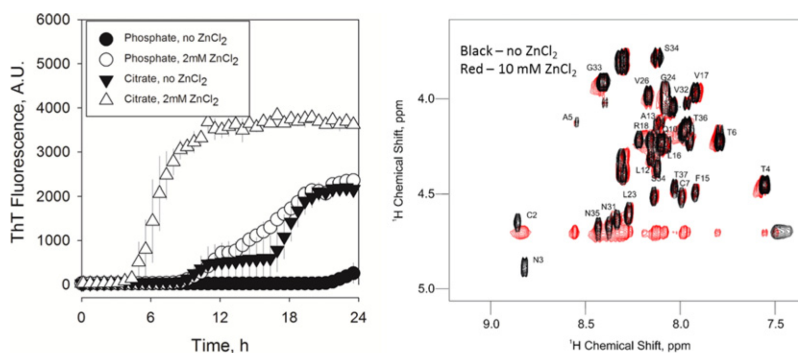
[b] Laboratory for Macromolecules (LAMAC-DIMAV), Brazilian National Institute of Metrology, Quality and Technology - INMETRO, Av. N. Sa. das Graças, 50 - Xerém, Duque de Caxias-RJ, 25250-020 Rio de Janeiro, Brazil

[c] National Institute of Science and Technology for Structural Biology and Bioimaging (INBEB-INCT), Federal University of Rio de Janeiro, Rio de Janeiro 21941-590, Brazil

HIGHLIGHTS

- Zinc interacts with murine amylin as measured by zincon, SPR, fluorescence, IMS, NMR.
- Zinc is a heterotrophic modulator of murine amylin assembly and aggregation.
- Zinc regulation of amylin does not require the His residue.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 12 June 2016
Received in revised form 10 September 2016
Accepted 17 September 2016
Available online 20 September 2016

Keywords:
Amylin
Islet amyloid polypeptide
Zinc
Amyloid
Diabetes
Oligomer
Islet

ABSTRACT

The secretory granule of the pancreatic β-cells is a zinc-rich environment copopulated with the hormones amylin and insulin. The human amylin is shown to interact with zinc ions with major contribution from the single histidine residue, which is absent in amylin from other species such as cat, rhesus and rodents. We report here the interaction of murine amylin with zinc ions *in vitro*. The self-assembly of murine amylin is tightly regulated by zinc and pH. Ion mobility mass spectrometry revealed zinc interaction with monomers and oligomers. Nuclear magnetic resonance confirms the binding of zinc to murine amylin. The aggregation process of murine amylin into amyloid fibrils is accelerated by zinc. Collectively these data suggest a general role of zinc in the modulation of amylin variants oligomerization and amyloid fibril formation.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Amylin, also known as islet amyloid polypeptide (IAPP), was discovered from pancreatic deposits of amyloid material from feline and human pancreas [1,2]. Amylin exert key physiological roles such as

Abbreviations: IAPP, islet amyloid polypeptide; ESI-IMS-MS, electrospray ionization–ion mobility spectrometry–mass spectrometry.

* Corresponding author at: School of Pharmacy, Federal University of Rio de Janeiro – UFRJ, CCS, Bss24, Ilha do Fundão, 21941-590, Rio de Janeiro, Brazil.

E-mail address: LML@UFRJ.BR (L.M.T.R. Lima).

http://dx.doi.org/10.1016/j.bpc.2016.09.008
0301-4622/© 2016 Elsevier B.V. All rights reserved.

inhibiting glucagon and somatostatin, reduction in glycemia, gastric emptying, lactate production, and glucose and glycogen metabolism in muscle [3,4]. Amylin is co-secreted along with insulin by the β-cells in the Langerhans islets [5,6], though also found in some non-β-cells [7]. Amylin and insulin are differently expressed and metabolized [8–10].

The presence of amyloid deposits correlates with loss of β-cells in humans [11–14]. Prefibrillar oligomers, found accumulated *in vivo* in the pancreas of both diabetic and non-diabetic humans [13,15,16], have implicated as potentially toxic forms of amylin [15,17] although their exact nature has been subject to controversy [17–23]. However, the understanding of the molecular basis of amylin dysfunction and the progression of amyloidosis along with diabetes *in vivo* has been limited, since toxic material in pancreatic islets of the most common experimental animal models such as mice and rats (their amylin sequences are the same) have not been consistently documented. The lack of a reliable murine model for amylin-related amyloid pathologies prompted the early development of transgenic models of mice overexpressing human amylin [24–30] (http://jaxmice.jax.org/strain/008232.html), which spontaneously develop diabetes and amyloid pancreatic deposits as a function of aging [31,32]. Evidences have also risen for aging as a risk factor for the appearance of pancreatic toxic species in and humans [13].

A landmark study have shown that the proline-rich amylin isoforms, such as murine amylin, are more water soluble [33], and would not be prone for aggregation *in vitro* or *in vivo*. This triple-proline hypothesis, supported by the lack of detection of amyloid fibrils *in vivo*, became widely accepted. The immediate benefit of the physico-chemical features of the proline-rich amylin variants was soon recognized, giving place for the development of the triple-proline human amylinomimetic compound ($Pro^{25,28,29}$; named pramlintide) currently available for therapeutic purposes. However, even pramlintide can form amyloid fibrils [34]. Murine amylin has also been shown to form soluble oligomers and amyloid fibrils in the absence of other cofactors *in vitro* [35], with morphologic properties similar to human amylin. This was the first time that high order oligomers of murine amylin were reported by extensive morphologic and biophysical techniques, including demonstration of the sequential oligomerization by the use of high definition electrospray ionization - ion mobility spectrometry - mass spectrometry (ESI-IMS-MS), subsequently confirmed independently by a similar approach [36,37]. In fact, several studies have long shown aggregation and amyloid-like tinctorial properties for murine amylin *in vitro* [34, 38,39], including a pH-dependence similar to human amylin [40], although they all have been assumed to be of minor importance.

Despite the intensive research in the field, the molecular mechanism for amylin oligomerization and the formation of amyloid deposits in pancreas is still not understood. Changes in overall metabolism in β-cells [41–45] as well as in the proteosthasis, including hyperinsulinemia and hyperamylinemia [16,46] may pose a disturbed interactome scenario paving the route towards the toxic aggregation. Some factors that would influence the amylin aggregation due to their immediate relation to amylin physiologic environments – including secretory granule - are lipid interfaces [47,48], unbalanced interaction with soluble or crystalline insulin [49–53], pH [40,54–56], modulation of insulin-degrading enzyme (IDE) [57,58], amyloid beta [59–62] or metals [63–68], including zinc [69]. In this process, the resulting complexity makes the deconvolution of multiple risk factors in amyloid development a difficult task, would require a robust *in vivo* model with minimal intervention.

The secretory granules of the pancreatic β-cells comprise high content of insulin and other elements, such as calcium, magnesium, potassium, sulfur, phosphorus – most likely phosphate - and zinc. [70]. Zinc (zinc ion, Zn^{2+}) is complexed by the crystalline insulin [71], which becomes non-crystalline upon depletion of zinc as shown in mices knockout for the zinc transporter ZnT8 [72]. Zinc has also been shown to bind and regulate interconversion of human amylin [73]. The His18 in human amylin is corresponds to the Arg18 in the murine amylin, which lacks His residues in its sequence. Since the zinc interaction with the murine amylin have preliminarily ruled out due to the lack of signal in calorimetric assays [67], the His18 has been attributed to provide the main driving force in zinc binding by human amylin. In the context of the correlation of lower dietary zinc with increased diabetes risk [74], the function of the vesicular zinc transporter ZnT8 (gene SLC30A8), and its relationship with diabetes incidence [75–77], a major role of zinc in the regulation of human amylin interconversion, formation of toxic species and amyloidogenesis has been proposed and motivating deeper investigation on this subject [67,69].

The His residue is not the only requirement for zinc coordination by proteins, since other aminoacids can fulfill the coordination sphere of varying metal ions [78–80]. The hydroxyl of tyrosine, the carbonyl oxygen of the peptide backbone or from the asparagine or glutamine can also provide stable zinc complexes [81]. In view of the fact that amylin-derived amyloid deposit can also be found in animals whose amylin sequence are also deprived of His residues, such as cat [2,31], rhesus monkey [82], pig [83], dog [84] and bear [85] (Supp. Fig. 1 – Alignment), we have investigated whether a His residue is necessary for zinc binding and regulation of murine amylin aggregation.

2. Material and methods

2.1. Materials

High-purity (>95%) carboxy-amidated murine (CAS 124447-81-0) and human (CAS 122384-88-7) amylin, with a C2–C7 disulfide bond was purchased from Genemed Biotech Inc. (CA, USA). The identity of the amylin peptides was confirmed by mass spectrometry (ESI-IMS-MS and MALDI-ToF-MS) performed both by the CRO and independently by our own group. Stock amylin solutions were prepared at 10 mg/mL (2.55 mM) in DMSO and stored at 4 °C. All other reagents were of analytical grade.

2.2. Zinc displacement assay

The interaction of murine amylin with zinc was assessed by the Zincon:Zn displacement assay [86,87]. 500 mM Zincon was incubated in untreated, transparent 96-well flat-bottom plate with 250 μM $ZnCl_2$ and varying concentration of murine amylin in 10 mM Tris.Cl pH 7.4, incubated at 25 °C for about 30 min and the absorbance at 620 nm was measured in a Spectramax M5 (Molecular Devices) microplate reader. The conversion from absorbance to concentration of zinc:zincon complex was performed by using an analytical curve obtained from measuring A620 nm of zincon solution in the presence of varying concentration of $ZnCl_2$ ($r^2 > 0.99$).

2.3. Isothermal binding assays

Murine amylin was labeled with fluorescein isothiocyanate (FITC; Sigma-Aldrich; 0.2 mM final concentration, from stock in DMSO at 5 mM freshly prepared) in 20 mM Na_2HPO_4, 300 mM NaCl, pH 7.0, for 60 min at 25 °C protected from light. The reaction was quenched by adding an excess of Tris.Cl. The free fluorescein was removed by chromatography with a Sephadex G25 desalting column (GE Healthcare, Brazil). The labeling efficiency, final yield and protein quantification were calculated as described elsewhere [88]. The labeling efficiency was below 1 mol FITC:1 mol peptide (typically 0.6 mol/mol) for all labeling procedures. The labeled amylin was stored until use protected from light at −20 °C for no longer than 3 months.

The isothermal binding assays were conducted in 96-well flat-bottom black plate (Corning #3915) using 50 nM FITC-labeled murine amylin as tracer and varying concentration of murine amylin in the indicated amount of $ZnCl_2$. The buffer composition was as indicated in their respective figure legend. Measurements were performed in a Spectramax M5 (Molecular Devices) microplate reader, with excitation

Download English Version:

https://daneshyari.com/en/article/5370717

Download Persian Version:

https://daneshyari.com/article/5370717

Daneshyari.com