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Aggregation of the diabetes-related peptide ProIAPP$_{1-48}$ measured by dynamic light scattering

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ABSTRACT

Islet amyloid polypeptide (IAPP$_{1-37}$) or amylin is implicated in the aetiology of diabetes. It is found as amyloid along with its precursor ProIAPP$_{1-48}$ in the islets of Langerhans in the pancreas. Metals have been implicated in amyloidogenesis of both IAPP and ProIAPP. Herein we have used dynamic light scattering (DLS) to investigate how Al(III) and Cu(II) influence aggregation of ProIAPP$_{1-48}$ under near-physiological conditions and in a biologically-relevant timeframe. ProIAPP$_{1-48}$ formed primarily sub-micron particles within 5 min (e.g. 470 nm at 15μM peptide) that grew to micron-sized particles (1310 nm) within a 30 min timeframe. Equimolar Al(III) had little influence upon particle size at either 5 (656 nm) or 30 min (1250 nm) while Cu(II) tended to increase particle size over the same time period (731–1300 nm). It is suggested that any effects of Al(III) and Cu(II) reflected their well known tendencies to support β-sheet or amorphous aggregates of ProIAPP$_{1-48}$ respectively.

1. Introduction

Amylin or IAPP is a peptide composed of 37 amino acids that was first discovered as a constituent of amyloid deposits in the islets of Langerhans in individuals diagnosed with diabetes [1]. IAPP is highly amyloidogenic and it is this property that implicates it in the degeneration of islet β cells in diabetes [2]. The precursor to IAPP is the 67 amino acid peptide ProIAPP that upon incomplete processing leads to ProIAPP$_{1-48}$ which has also been found in amyloid deposits in diabetes [3]. Recent research implicates aberrant or incomplete processing of ProIAPP in the aetiology of diabetes [4].

While both IAPP and ProIAPP$_{1-48}$ readily form amyloids in vitro their concentrations in vivo are significantly below saturation and a burgeoning body of research is investigating this conundrum [5]. The aggregation of super-saturated concentrations of IAPP is influenced by aluminium [6,7], iron [7], zinc [7,8] and copper [7]. It remains equivocal as to whether Al(III), Fe(III) and Zn(II) promote amyloid (β sheet) formation while it is clear that Cu(II) prevents IAPP from assembling into β sheet structures [7] as recently confirmed [9–13]. ProIAPP$_{1-48}$ forms amyloid less readily than IAPP and while there are few data on its interactions with metals it is also the case that Cu(II) prevents ProIAPP$_{1-48}$ from forming β sheets of amyloid [12,14,15].

It is widely believed that the cytotoxicity's of IAPP and ProIAPP$_{1-48}$ is related to their propensity to form toxic oligomers during the early stages of amyloid formation [5] and it has been suggested that metals and specifically Cu(II) potentiate toxicity through stabilisation of these oligomeric forms [11,16]. We have used dynamic light scattering to investigate in vitro how Al(III) and Cu(II) influence the immediate aggregation of ProIAPP$_{1-48}$ under near physiological conditions.

2. Materials and methods

2.1. Synthesis and purification of peptide

ProIAPP$_{1-48}$ fragments were synthesised using an Applied Biosystems 433 A peptide synthesiser through the application of standard FMOC-based solid phase methodology. Purification of the peptide was performed using RP HPLC on a POROS 20R2 column using water/acetonitrile mixtures buffered with 0.1% TFA. The peptide content of the purified material (77%) was determined by quantitative amino acid analysis and lyophilised aliquots were stored at −80 °C prior to the preparation of peptide stock solutions.

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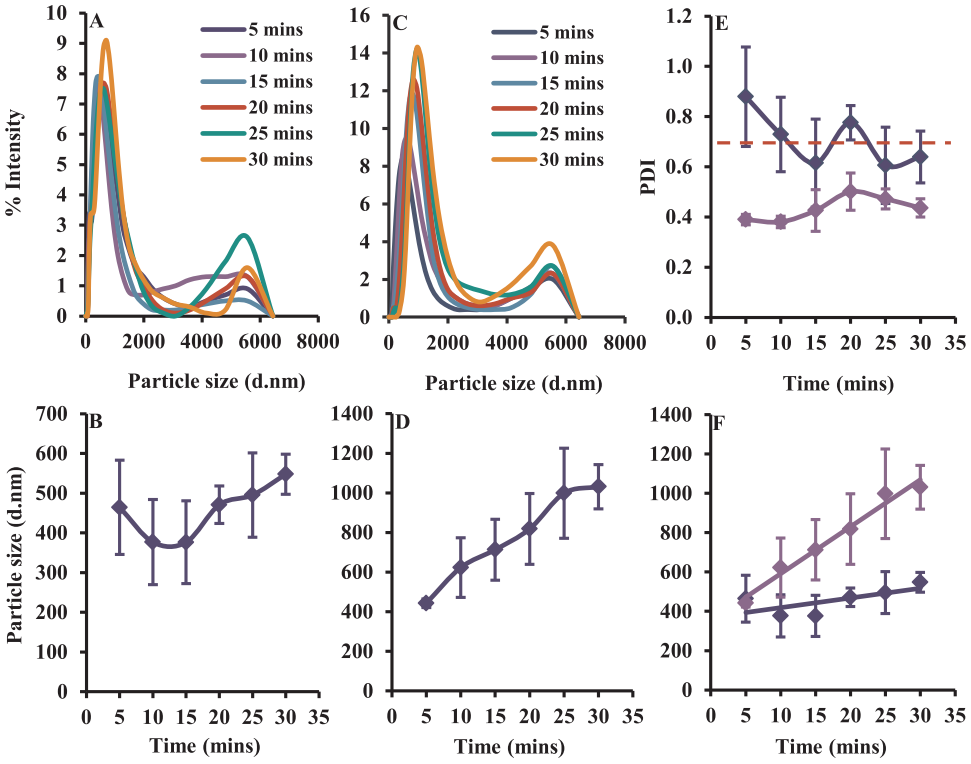

Fig. 1. DLS analyses of ProIAPP$_{1-48}$ in KH buffer over 30 min at 37 °C. Figures A & C represent the raw intensity distributions (d.nm) generated through the measurement of 30 & 60 μM peptide respectively. Figures B & D show the average median particle size (d nm) for 30 & 60 μM peptide respectively. Figures E & F show the PDI values and comparative analysis of the average median particle size data (d.nm) respectively for 30 & 60 μM peptide (blue and purple lines respectively). Error bars represent the ± SD of the measurement where n = 3. The red dotted line on Fig. E illustrates the PDI limit (0.7) over which particle size measurements become unreliable. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

2.2. Preparation of peptide stock solutions

Peptide stocks were prepared to a final concentration of 1 mg/mL (*ca* 200 μM) via the addition of ultrapure water (< 0.067 μS/cm) to thawed peptide lyophilisates. This stock was then used to prepare smaller individual volumes of the peptide in order to achieve the final concentrations of peptide included in the following experiments and these aliquots were stored at −20 °C until required.

2.3. Preparation of treatments

To observe the aggregation behaviour of ProIAPP$_{1-48}$ under relevant physiological conditions, thawed peptide aliquots were introduced into two modified Krebs-Henseleit (KH) buffers (pH 7.4 ± 0.05), one containing an absence of citrate and the other supplemented with 1 mM citrate. The final concentrations of peptide included in these treatments were 15, 30 & 60 μM. These represent a range of concentrations from below saturation to concentrations where ProIAPP$_{1-48}$ is known to form amyloid [15]. Treatments containing equimolar concentrations of metal ions (Al^{3+} & Cu^{2+}) were prepared via the addition of certified metal stock solutions (Perkin Elmer, UK) to the KH buffer prior to the introduction of the peptide.

2.4. Particle size analysis

ProIAPP$_{1-48}$ aggregation in the absence and presence of metal ions was monitored by dynamic light scattering (DLS) using a Zetasizer Nano ZS equipped with a 633 nm laser (Malvern Instruments, UK). Samples were prepared in a pre-rinsed quartz low volume cuvette (100 μL) and analysed at 37 °C for 30 min over a range of 0.1–6 μm. Scattered light was detected at an angle of 173° (backscatter) and three measurements consisting of 10 runs each (10 s per run) were conducted per individual sample. These machine replicates were averaged thereafter to obtain the mean distributions generated over a 5-min block and the d50 of these distributions was used in the final analysis. The data shown in the following figures represents the average median particle size of three or six individual sample replicates.

Samples which did not generate enough photonic scattering to generate a reliable distribution (mean count rate < 100 kcps) were deemed to be free of particulates. To ensure the quality of the distributions obtained (see autocorrelation data for all treatments in Supplementary), all accepted measurements had a PDI and multimodal fit error of < 0.7 & < 0.005 respectively, unless otherwise indicated. Measurements of this nature were accepted as of "good" quality in direct accordance with the QC parameters included within the Malvern Zetasizer software.

The size of peptide aggregates was determined using the Stokes-Einstein equation that describes the relationship between hydrodynamic diameter (dh) and velocity of particles within the system undergoing Brownian motion (D) (Eq. (1)).

$$Dh = kT/3\pi\eta D \quad (1)$$

Distributions were generated using a non-negative least squares fit (NNLS) of the correlation function followed by L-curve regularisation.

2.5. Statistical analysis

Statistical analysis of the data was performed using GraphPad Prism software (v.7). The normality of datasets was determined using a Shapiro-Wilk test and those which yielded a p value ≤0.05 were analysed for significance using non-parametric tests. Comparisons over time were performed using a repeated measures ANOVA with a Geisser-Greenhouse correction followed by Tukey post hoc tests or a non-parametric equivalent (Friedman test followed by Dunn post hoc tests). Comparisons between peptide concentration or presence/absence of metals were performed using an ordinary ANOVA followed by Tukey post hoc tests or a non-parametric equivalent (Kruskal-Wallis test followed by Dunn post hoc tests). Comparison of the relative rate of aggregation between samples was performed using linear regression analysis. P values ≤0.05 were considered to be statistically significant.