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Aluminium, β-amyloid and non-enzymatic glycosylation

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Abstract The non-enzymatic glycosylation of β-amyloid is implicated in the aetiology of Alzheimer's disease. However, controversy surrounds the nature of any involvement and a potential mechanism has not been fully elucidated. We present evidence of an aluminium-induced aggregation of the AβP(25–35) peptide and speculate that the mechanism of formation of our ordered β-amyloid aggregates might involve non-enzymatic glycosylation and/or site-specific crosslinking of β-amyloid fibrils by atomic aluminium.

Key words: Aluminium (biology); Beta-amyloid; Alzheimer's disease; Non-enzymatic glycosylation

1. Introduction

Recent research has implicated the process of non-enymatic glycosylation in the aetiology of Alzheimer's disease (AD) [1,2,3]. The research has generated considerable discussion in the scientific literature [4,5] as to the role this process might play in the formation of neurofibrillary tangles and senile plaques, the characteristic pathological structures associated with AD. The most recent opinion suggests that the glycosylation of β-amyloid (and tau) occurs only after crosslinking of the peptide and any subsequent related neuronal damage [5]. The evolutionary disadvantage of glycosylated protein, such that long-lived proteins would not be expected to have exposed glycosylation sites [6], could be interpreted to support the latter assertion. However, if the biochemical environment in the immediate vicinity of a protein were to change so as to irreversibly alter the conformation of that protein then the susceptibility of the protein to non-enzymatic glycosylation could change. We have shown that aluminium is an important influence on the conformations of β-amyloid peptides [7] and in the research detailed herein have tested the hypothesis that aluminium would promote the non-enzymatic glycosylation of AβP(25–35) by inducing a conformational change in the peptide structure and thus exposing the glycosylation site on the peptide.

2. Experimental

Synthetic AβP(25–35) was obtained from Bachem Inc. (CA, USA) and dissolved in ultrapure water to give a stock solution of 0.23 mmol·dm^{-3}. Peptide was then dissolved in NaPIPES buffer (Aldrich, UK) at pH 6.0, and containing 5 mmol·dm^{-3} glucose (Sigma, UK), to give a nominal working peptide concentration of 10 μmol·dm^{-3}. Aluminium was added to half of these solutions from an acidified 37 mmol·dm^{-3} $Al(NO_3)_3 \cdot 9H_2O$ stock (Perkin-Elmer Certified Stock) to give a final concentration of 10 μmol·dm^{-3} total aluminium. Experimental solutions were maintained at 25°C in an incubator for up to 8 weeks. One hour after the preparation of these solutions and every week thereafter for up to 8 weeks each solution was analysed spectrofluorimetrically (Perkin-Elmer LS50) for the formation of Schiff bases (an early indicator of glycosylation [8]) and to ascertain the biological availability of the added aluminium. The extent of Schiff base formation was shown by emission spectra with maxima in the range 400–450 nm upon excitation of the solutions at 360 nm. The biological availability of the aluminium in each solution was ascertained upon the addition of the fluor Morin (Sigma, UK), excitiation at 420 nm giving emission maxima in the wavelength range of 490–510 nm. This method is described in full in a paper presently in preparation.

After 8 weeks, samples from each solution were applied to carbon-coated Formvar on 200 mesh copper grids, dried, washed with 2% uranyl acetate and viewed in a JEOL 100CX electron microscope.

3. Results and discussion

We were not able to identify the occurrence of glycosylation in either the presence or absence of aluminium. There were increases in the fluorescent signal in both treatments over the 8 week incubation period and the increase was greater in the presence of aluminium than when aluminium was absent. However, all of these changes in the fluorescent signal were found to be within the 95% confidence intervals. This lack of statistical significance does not preclude the possibility that glycosylation reactions occurred in either solution as, in hindsight, it is possible that our sampling interval was too long (1 week) since Schiff bases formed during glycosylation reactions are transient forms and may rearrange to give Amadori-like products within a few days of their formation [1].

The application of Morin as an arbitrary indicator of biological availability revealed an interesting result. We found that the influence of the glucose in the solution to which aluminium was added was to promote a high and sustained biological availability of aluminium. The biological availability of aluminium was increased five-fold compared to several glucose-free control solutions. This influence of glucose on the biochemistry of aluminium has not hitherto been reported. To ascertain what, if any, effect this unusual aluminium chemistry might have on the morphology of the β-amyloid in solution we used electron microscopy to look for the formation of β-amyloid fibrils. In the absence of added aluminium we observed distinct, relatively short, amyloid fibrils of approximately 7–8 nm in diameter (Fig. 1a). These single β-amyloid fibrils were completely absent from the solutions containing aluminium and had been replaced by aggregates made up of fibrils of approximately 9–10 nm in diameter (Fig. 1b,c). These structures were considerably longer than the single fibrils formed in the absence of aluminium and the individual fibrils were spirally wound around each other in a paired helical filament-like arrangement.

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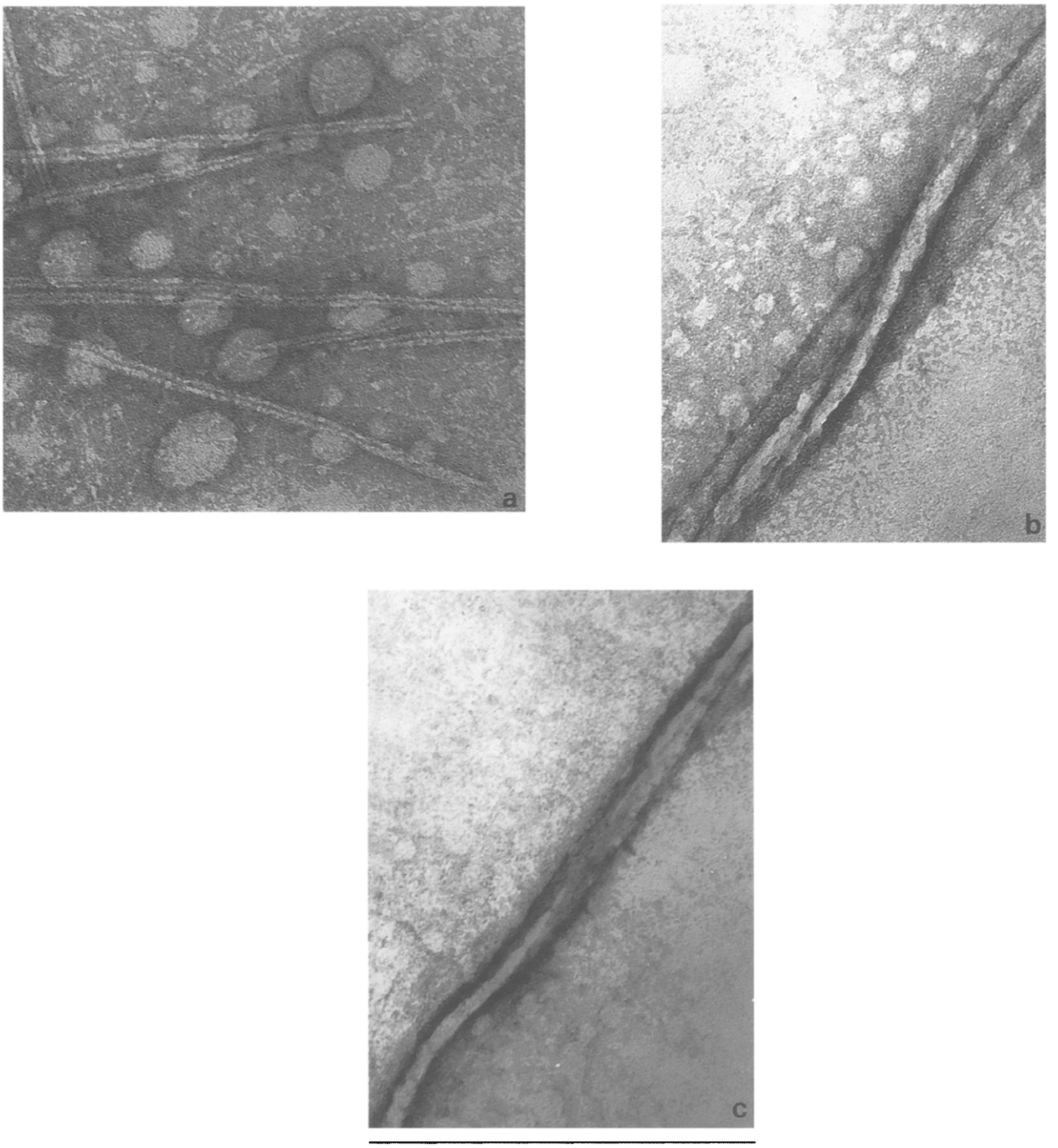

Fig. 1. (a–c) Electron micrographs of AβP(25–35) in the (a) absence and (b,c) presence of 10 μmol·dm^{-3} total aluminium. Magnification is 98,000× in each plate.

The aluminium-induced aggregation of the longer β-amyloid peptides [9,10] and the amyloid precursor protein [11] has been reported previously. However, in these studies it was neccessary to add very high concentrations of aluminium (and sometimes peptide) before aggregation of the peptide could be determined. Where electron microscopy was undertaken the amyloid was found in amorphous aggregates and not in the ordered structures found in the research detailed herein. It is possible that in these earlier studies amorphous aluminium hydoxide acted as a template or seed for the disordered aggregation of the peptide, whereas in the present research, the presence of glucose prevented the formation of such large agglomerates of aluminium hydroxide and, presented conditions which promoted the formation of ordered helical structures of β-amyloid either through the promotion of non-enzymatic glycosylation or through specific cross-linking reactions involving atomic aluminium.

In conclusion, whilst non-enzymatic glycosylation might not

be expected to be a precursor to the formation of an AD-like pathology it is conceivable that small changes in the biological environment of a peptide or protein could implicate this process in the aetiology of such a disease state. Strong precedents for such already exist in diabetes research [12,13].

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