Amorphous aluminosilicates promote nucleation of amyloid β protein and tachykinins

John M. Candy, Arthur E. Oakley and James A. Edwards

MRC Neurochemical Pathology Unit, Newcastle General Hospital, Newcastle upon Tyne NE4 6BJ

One of the hallmarks of Alzheimer’s disease is the deposition of amyloid in senile plaques. The major constituent of senile plaque amyloid is a 4.5kDa protein, amyloid β protein, which contains 42 or 43 amino acids (reviewed in 1). There have been several reports of aluminosilicate associated with senile plaques in Alzheimer’s disease [reviewed in 2] and it has been reported that a focal accumulation of aluminium in the form of aluminosilicate is present in the core of human senile plaques in Alzheimer’s disease, Down’s syndrome and in normal elderly subjects [3,4]. The core of mature senile plaques consists of a compact, roughly spherical accumulation of radiating bundles of amyloid β protein fibrils. The ‘starburst’ appearance of senile plaque cores at the ultrastructural level (Fig 1A), is reminiscent of protein crystal growth. A range of minerals have previously been shown to promote nucleation of protein crystals [5]. The effect of amorphous aluminosilicates on substance P, eldeanoin and synthetic amyloid β protein has therefore been investigated since these peptides have previously been shown to form linear polymeric fibrils in aqueous solution with a β-sheet secondary conformation [6,7].

Suspensions of 1mg substance P (Sigma), 1mg eldeanoin (Sigma) and 300μg of synthetic human amyloid β protein 1–42 were prepared in 1ml of ultrahigh purity distilled water in the absence of amorphous aluminosilicate and with 200μg precipitated aluminium silicate (Merck) or 200μg magnesium trisilicate (Merck). The solutions were allowed to stand for up to 48h at ambient temperature. Five and 10μl aliquots of each solution were centrifuged (100,000g for 20min) onto 300 mesh grids that had been precoated with formvar and carbon. Polymeric peptide structures were visualised by negative staining with 1% uranyl acetate in water and examined using a transmission electron microscope fitted with an energy dispersive X-ray spectrometer.

Suspensions of both substance P and eldeanoin in distilled water in the absence of aluminosilicate contained linear, non-branched fibrils of indefinite length, with diameters ranging from 7–9nm (Fig 1B). The fibrils appeared on the grid as a random network of individual fibrils, without evidence of any marked lateral aggregation. In the presence of aluminosilicate both substance P and eldeanoin suspensions showed fibrillary aggregates that had a stellate appearance (Fig 1C). Energy dispersive X-ray microanalysis showed the presence of an amorphous silicate in the most densely packed aggregations. In more loosely packed aggregations individual substance P and eldeanoin fibrils appeared to emanate from the surface of the aluminosilicate particles. Suspensions of amyloid β protein in distilled water in the absence of aluminosilicate also showed the presence of linear, non-branched fibrils of indefinite length with a diameter of 4–6nm (Fig 1D). The appearance of the amyloid β protein fibrils was very similar to that described previously for synthetic amyloid β protein 1–43 [7]. Individual amyloid β protein fibrils were randomly distributed with no evidence of aggregation. In the presence of aluminosilicate, aggregations could be observed and the individual fibrils appeared to originate from the surface of the aluminosilicate particles (Fig 1E). There was no evidence on the grid of amyloid β protein fibrils that were not associated with aluminosilicate particles. In contrast, the distribution of amyloid β protein fibrils in the presence of magnesium trisilicate was very similar to that observed in distilled water alone (Fig 1F).

These results show that amorphous aluminosilicates can promote nucleation and aggregation of both tachykinins and amyloid β protein. This suggests that aluminosilicates may play a role in the nucleation of amyloid β protein and give rise to the characteristic ultrastructural appearance of senile plaque cores.

We thank Dr. R. Jakes, Laboratory of Molecular Biology, Cambridge for providing synthetic amyloid β protein 1–42.