Aggregation of Alzheimer’s peptides.

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Two pathogenic mutations in the gene encoding the amyloid precursor protein (APP) are associated with the deposition of amyloid of the β/A4 type [1] in the walls of leptomeningeal and cerebrocortical blood vessels. The replacement of glutamate (E) by glutamine (Q) at position 618 of the 695 isoform of APP, which corresponds to residue 22 of β/A4, has been associated with cerebral amyloid angiopathy-Dutch type [2,3]. This rare autosomal dominant disease results in recurrent cerebral haemorrhage in middle age [4]. A mutation at the adjacent residue 21 of β/A4 (glycine instead of alanine) has been identified in one Dutch family with a history of both pre-senile dementia and cerebrovascular amyloidosis [5]. The pathogenic mechanisms of these mutations are unknown.

It is well known that synthetic peptides corresponding to full length and fragments of β/A4 aggregate into fibrils with the physical characteristics of amyloid. An increased rate of fibril formation has been observed in fragments containing the Q22 mutation although the rate of aggregation was not measured [6]. We have monitored the aggregation of synthetic peptides corresponding to residues 13-26 of β/A4 to determine if the G21 and Q22 mutations affect the rate of fibril formation of these particular fragments. The peptides studied were Ac-HHQKLVFFAEDVGS-NH₂ (P11.26G21), Ac-HHQKLVFFAQDVGS-NH₂ (P11.26Q22) and Ac-HHQKLVFQGDVGSS-NH₂ (P13.26G21). The aggregation of the peptides at several concentrations from 500mM Tris/HCl, pH 7.4 at 37 °C was monitored by measuring the amount of peptide remaining in solution at various time points (Fig.1 legend). Fig.1 shows the results at 0.2mg/ml and 1mg/ml. At 1mg/ml, 60% of β11.26 and 75% of β11.26G21 remained in solution after 24 hours, whereas β11.26Q22 had completely precipitated from solution by this time. Negative stain electron microscopy revealed that the three peptides precipitated as fibrils (diameter 7-15nm) (Fig.2). When stained with Congo Red, they all exhibited green-red birefringence, which is characteristic of amyloid.

These results, together with those obtained at intermediate concentrations (0.5mg/ml and 0.8mg/ml), demonstrate that the rate of aggregation of β11.26Q22 is dependent on concentration within this range. Results over a similar range reveal that concentration has some influence on the rate of aggregation of β11.26. However, in the case of β13.26G21, there was no obvious affect of concentration.

Mixtures of β11.26 with β11.26Q22 or β11.26Q21 (0.5mg/ml of each in 50 mM Tris/HCl; pH 7.4 at 37 °C) were also studied. Our results (not shown) demonstrate that the rate of aggregation of β11.26Q22 is reduced in the presence of β13.26 (60% remaining after 24 hr) while β11.26G21 remains unaffected. β13.26 aggregated faster in the presence of β11.26Q22 (60% remaining in solution after 24 hr) than in the presence of β13.26G21 (90% remaining in solution).

Our results support the previously reported observation by Wisniewski et al [6] that the Q22 mutation increases the propensity of the β/A4 peptide to aggregate into amyloid. The results for β13.26G21, however, suggest that the mechanism of pathogenesis of the G21 mutation is different. The G21 mutation may reduce the cleavage at the α-secretase site [7] resulting in an increased level of processing of APP to β/A4 (possibly at the β-secretase site [8]). Alternatively, it may make β/A4 more resistant to the normal pathways of degradation.

We wish to thank Research Into Ageing and The Wellcome Trust for financial support, The Department of Education for Northern Ireland for a Postgraduate Studentship (AC) and Pat Larkin and Orla O’Shea, QUB, for help with the electron microscopy.


Fig. 1.Effect of concentration on the rate of aggregation at (a) 0.2mg/ml and (b) 1mg/ml. Peptides were incubated at the indicated concentration. The amount of peptide remaining in solution was measured using reverse phase HPLC.

Fig. 2 Electron micrograph of β13.26 fibrils stained with 2% uranyl acetate. Magnification = 70000.