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β-Cyclodextrin interacts with the Alzheimer amyloid β-A4 peptide

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Abstract

Electrospray ionisation mass spectrometry has been used to show that the synthetic 40 amino acid β-amyloid peptide (β1–40) interacts with the cyclic oligosaccharide β-cyclodextrin. This interaction, presumably with the hydrophobic aromatic moieties on the peptide, has been shown to diminish substantially the neurotoxic effects of β1–40 in a cell line.

Key words: Amyloid; A4 peptide; Alzheimer's disease; β-Cyclodextrin; Electrospray ionisation mass spectrometry; Fibrillisation

1. Introduction

Alzheimer's disease is characterised pathologically by the classical senile plaque in the human brain. This consists of a central core surrounded by dystrophic neurites, reactive microglia and astrocytes. The major constituent of the plaque core has been shown [1,2] to be the β-A4 amyloid peptide, containing 39–43 amino acids (Fig. 1). The function of β-amyloid is poorly understood. It has been shown, as least in vitro, to possess both neurotoxic and neurotrophic properties, and it may also function as an adhesion molecule [3]. Studies in vitro have reported neurotoxicity in cultured neuronal preparations [4,5] and in cell lines [6]. Early controversy regarding whether β-amyloid was neurotoxic in vitro appear to have been resolved by 'ageing' studies. After 2–4 days preincubation, β-A4 fibrillises and is neurotoxic to rat hippocampal neurones, whilst in its freshly solubilised state the material is lacking in toxicity and may even promote neurite outgrowth [4]. The adoption of a β-sheet conformation is thought [7] to be essential for aggregation and fibrillisation of β-A4. It has also been suggested that it is the interaction between the hydrophobic residues of β-A4 that is the primary mechanism of the aggregation process [7]. Reduction in the interaction of the residues could ultimately be an important factor in controlling the progression of Alzheimer's disease.

We have shown recently [8] that peptides containing aromatic acid residues, in particular tryptophan and phenylalanine, interact with β-cyclodextrin. Encapsulation of such residues by this water-soluble oligosaccharide can alter both the secondary structure of peptides and their hydrophobicity characteristics.

It is well known [9] that the B-chain of insulin is involved in the formation of dimers and higher polymers. This chain contains three phenylalanine and two tyrosine residues in comparison to the A-chain which only contains two tyrosine residues. It is of interest that the addition of hydroxypropyl-β-cyclodextrin has been reported [10] to prevent the aggregation of insulin in solution at ambient temperatures. Such an inhibition may well be due to conformational changes in the insulin as a result of complexation with the cyclic oligosaccharide.

In the present study, we examine the ability of the 40 amino acid β-amyloid protein (β1–40) to interact with β-cyclodextrin and we investigate if this interaction can control the neurotoxic effects of the peptide.

2. Materials and methods

2.1. Mass spectroscopy studies

A solution (100 μl) of β1–40 in water:methanol, 1:1 (v/v), containing 5% acetic acid (v/v) (0.5 mg/ml) was added to a solution (100 μl) of β-cyclodextrin in water (0.16 mM, about 0.185 g/l). An aliquot of this mixture (50 μl) was injected into a flow of water:methanol, 1:1 (v/v) (4 μl/min), and transported to an electrospray source fitted to a single stage quadrupole mass spectrometer (Vestec 201-A; Vestec Corp., Houston, TX). Ionisation was affected by applying 2.5 kV to the electrospray needle; spectra were recorded over a mass range of 2000 to 100 u with 20 s scans in profile mode.

2.2. Fibrillisation of β1–40 and neurotoxicity assay

The β1–40 used in the present experiments was Lot No. ZK600 purchased from Bachem UK Ltd. The peptide was initially dissolved in 0.1% acetic acid at 4.6 mM (2 mg/ml). Greater than 95% fibrillisation was induced by diluting with peptide solution with 4 vols. of 62.5 mM MES buffer, pH 5.75, and incubating at room temperature for 2 h (Wetzel, Wood, personal communication).

Where tested, β-cyclodextrin was present in the MES buffer at 15 mM. Following this fibrillisation step, the samples were diluted to

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1 5 10 15 20 25
D A E F R H D S G Y E V H H Q K L V F F A E D V G

30 35 40
S N K G A I I G L M V G G V V I A T

Fig. 1. Amino acid sequence of β-A4 peptide.

appropriate concentrations for the neurotoxicity assay in Dulbecco's Minimal Essential Medium (DMEM).

To assess neurotoxicity, PC12 cells (rat phaeochromocytoma; ECACC, Porton Down, UK) were plated at 6×10^4 cells/cm^2 in a 96-well microtitre plate (Nunc). After 2 h attachment, fresh DMEM containing β1–40, β-cyclodextrin or both, was then added. The highest concentration of β1–40 used was 23 μM (= 10 μg/ml): this contained, due to the dilution, 75 μM β-cyclodextrin. After a further 24 h, cell viability was assessed by an MTT assay, which depends upon the integrity of mitochondrial respiration for the conversion of the metabolic dye MTT to a blue formazan product.

The degree of fibrillisation was estimated by incubating the β1–40 solution, in the presence or absence of β-cyclodextrin, with 25 μM Congo red solution, pH 7.4, and calculating the amount of Congo red bound [11].

3. Results and discussion

The electrospray mass spectrum of synthetic β1–40 is shown in Fig. 2a. The two signals at values of *m*/2 of 1444.3 and 1083.5 correspond to the $[M + 3H]^{3+}$ and $[M + 4H]^{4+}$ forms of the peptide, respectively, and are consistent with a molecular weight of 4330. The spectrum reported [12] for the 42 amino acid peptide (dissolved in 0.1% trifluoroacetic acid in water) consists of two peaks corresponding to the $[A + 4H]^{4+}$ and $[A + 5H]^{5+}$ ions of this peptide. The difference in ionisation between the two peptides is probably due to the strength of the trifluoroacetic acid (used by the authors of [12]) which will generate more protonation and also destroy the conformation of the peptide. In the presence of β-cyclodextrin, β1–40 shows a number of additional peaks (Fig. 2b) related to complexation between the two molecules. The strongest signal at *m*/2 1367 corresponds to a 1:1 complex, $[A + CD + 4H]^{4+}$. In addition, a smaller peak related to the tri-ionised form $[A + CD + 3H]^{3+}$ is observed. Higher complexes of the forms $[A + 2CD + 4H]^{4+}$ and $[A + 3CD + 4H]^{4+}$ at *m*/2 1651 and 1834 are also seen. It is interesting that a penta-ionised species $[A + 3CD + 5H]^{5+}$ at *m*/2 1549 appears to be stabilised in the presence of the oligosaccharide.

β1–40 contains one tyrosine and three phenylalanine residues so that the main peptide–cyclodextrin complexes observed are almost entirely due to inclusion complexation of the hydrophobic aromatic moieties and the hydrophobic cavity of the cyclic oligosaccharide. Recently, we reported [8] the use of electrospray mass spec-

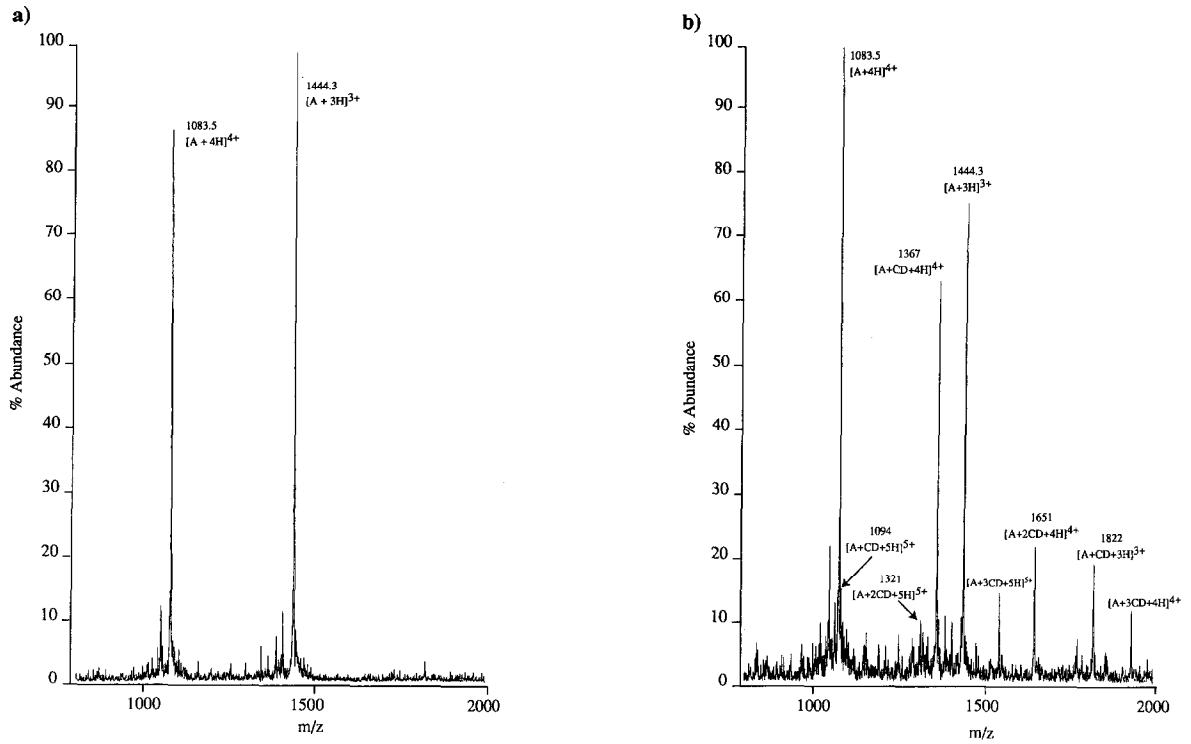

Fig. 2. Electrospray mass spectrum of synthetic β1–40 in (a) the absence and (b) the presence of β-cyclodextrin.

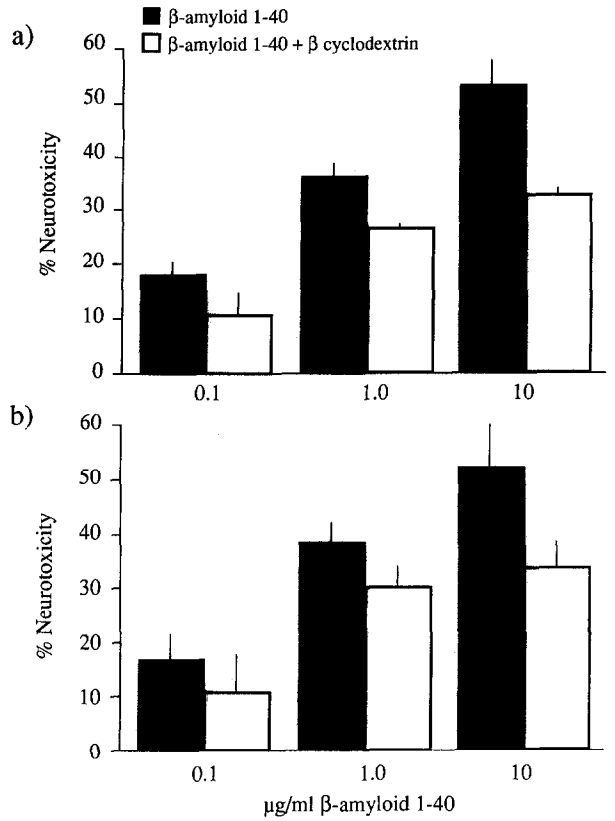

Fig. 3. Effects of β-cyclodextrin on neurotoxicity to β1–40 in PC12 cells. (a) 3×10^4 cells/cm^2 and (b) 6×10^4 cells/cm^2.

trometry to monitor the formation of such complexes for a number of peptides. We have now also found that minor signals could be the result of interactions between basic residues and the hydrophobic outer surface of the β-cyclodextrin. Such interaction can give rise to the formation of higher complexes. Having obtained mass spectrometric values for the interaction of cyclodextrin with β1–40, we investigated whether such an interaction could reduce or prevent the neurotoxic effects of this peptide. Samples of β1–40 were allowed to fibrillise in the presence and absence of β-cyclodextrin, as described in section 2. The results, at two different cell densities, are shown in Fig. 3. In the absence of β1–40, β-cyclodextrin had no effect on cell viability (data not shown). The presence of β-cyclodextrin during the fibrillisation time substantially reduced the cell loss induced by 0.23–23 μM β1–40.

In the absence of β-cyclodextrin, β1–40 which had been incubated firstly in MES buffer and subsequently with Congo red solution, bound 2.3 μM Congo red. When β-cyclodextrin (15 mM) was included in the MES buffer, only 0.1 μM of Congo red was subsequently bound. Although high concentrations of β-cyclodextrin do inhibit directly the binding of Congo red to fibrils, the effects observed in these present experiments are due to a prevention of fibril formation since the inclusion of an appropriate dilution of β-cyclodextrin in the Congo red solution after fibril formation only had a small effect on the Congo red binding (1.6 μM bound).

These data support the suggestion that β-cyclodextrin does indeed interact with β1–40. Moreover, it appears that as a result of such an interaction, a considerable reduction in the neurotoxic effects of the peptide are observed. It can be inferred, from these results, that an inhibition of the fibrillisation of β1–40 attenuates its neurotoxic effects.

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