Volume 324, number 3, 293–295 FEBS 12597 June 1993
© 1993 Federation of European Biochemical Societies 00145793/93/$6.00

An interaction of β-amyloid with aluminium in vitro

Christopher Exley[a], Nicholas C. Price[b], Sharon M. Kelly[b] and J. Derek Birchall[a]

[a]*Unit for Inorganic Chemistry and Materials Science, Department of Chemistry, Keele University, Staffordshire, ST5 5BG, UK* and [b]*Department of Biological and Molecular Sciences, University of Stirling, Stirling, FK9 4LA, Scotland, UK*

Received 21 April 1993

We have used circular dichroism spectroscopy to confirm that, in a membrane-mimicking solvent, AβP(1–40) adopts a partially helical conformation and we have demonstrated the loss of this structure in the presence of physiologically relevant concentrations of aluminium. This is the first evidence of a direct biochemical interaction between aluminium and β-amyloid and may have important implications for the pathogenesis of Alzheimer's disease

Aluminum (biology); β-Amyloid protein; Alzheimer's disease, Circular dichroism spectroscopy

1. INTRODUCTION

Controversy surrounds the involvement of both aluminium and β-amyloid in the aetiology of Alzheimer's disease (AD). The cerebral deposition of β-amyloid, and possibly aluminium, is an invariant feature of the disease; however, a mechanism that adequately links the presence of either β-amyloid or aluminium to the neuronal degeneration that is characteristic of AD has not been described. Both aluminium [1] and β-amyloid [2] are neurotoxic and, in both, cell death is linked with disruptions in calcium homeostasis [3,4]. Recent evidence that AβP(1–40) forms transmembrane calcium channels in vitro that are irreversibly blocked by aluminium [5] prompted us to investigate the effect of aluminium on the conformation of this peptide.

2. EXPERIMENTAL

Synthetic AβP(1–40) and AβP(25–35) were obtained from Bachem Inc (CA, USA) and solutions prepared as detailed below. AβP(1–40) peptide was dissolved in MilliQ water to give a nominal [peptide] of 1.39 mmol·dm^{-3} The dissolved peptide was then diluted with 10 mmol dm^{-3} Tris buffer, pH 7.0, to give a final stock solution concentration of 0.23 mmol·dm^{-3}. Prior to use the stock was incubated at 37°C for 24 h as per the manufacturers instruction. After incubation traces of insoluble material were removed by centrifugation. For circular dichroism (CD) analysis the peptide was added to a solvent of 60% TFE (Aldrich, NMR Grade) /40% 10 mmol·dm^{-3} Tris buffer at pH 7.0 to give a working [peptide] of 40 μmol·dm^{-3} Aluminium was added from a 5 mmol dm^{-3} $Al(NO_3)_3 9H_2O$ (Aldrich) stock solution to give final nominal stock concentrations of 0, 10, 20, 50, 100, 200, 250 and 300 μmol dm^{-3}. The AβP(25–35) peptide was immediately dissolved in a solvent of 60% TFE/40% Tris buffer at pH 7.0 to give a stock solution concentration of 0 23 mmol·dm^{-3}. The peptide was initially used at a final concentration of 40 μmol·dm^{-3}, however, because of the poor resolution of the spectra obtained at this concentration subsequent spectra were run at a [peptide] of 0 23 mmol·dm^{-3}. Aluminium was added as per AβP(1–40) to give final nominal concentrations of 0, 10, 20 and 50 μmol·dm^{-3} Concentrations of the peptide stock solutions were verified by measurements of tyrosine absorbance at 280 nm.

CD spectra of both peptides were recorded at 37°C on a JASCO J-600 spectropolarimeter using quartz cells of a 0 05 cm path length.

3. RESULTS AND DISCUSSION

The conformations, in a membrane-mimicking solvent, of the polypeptide fragments AβP(1–40) and AβP(25–35) were studied using CD spectroscopy. The spectrum obtained for AβP(1–40) (Fig. 1) was in close agreement with that reported for the similar AβP(1–42) peptide [6]; that for the AβP(25–35) peptide (Fig. 2) has not hitherto been reported. Using the values of θ_{208} [7], α-helical contents of 20% and 0% are obtained for AβP(1–40) and AβP(25–35), respectively. We have also used the CONTIN method [8] to analyse the spectra. This gave values for the α-helical content in close accord with the θ_{208} analysis (21% and 5% for AβP(1–40) and AβP(25–35), respectively). The β-sheet contents were 58% and 61% respectively, with the remainder (including β-turn and random coil) 21% and 34%, respectively. Although the exact values of the β-sheet and remainder contents should be treated with a degree of caution, in view of the uncertainty regarding the application of reference spectra that are based on sets of globular proteins to the analysis of small peptides [9], the α-helical contents of the peptides can be regarded as reliable. It is of interest that the spectrum of the peptide AβP(29–42) (which overlaps with AβP(25–35)) has been analysed in terms of a large β-sheet content (61%) [6] and

Correspondence address: C. Exley, Unit for Inorganic Chemistry and Materials Science, Department of Chemistry, Keele University, Staffordshire, ST5 5BG, UK. Fax: (44) (782) 715 944.

Published by Elsevier Science Publishers B V

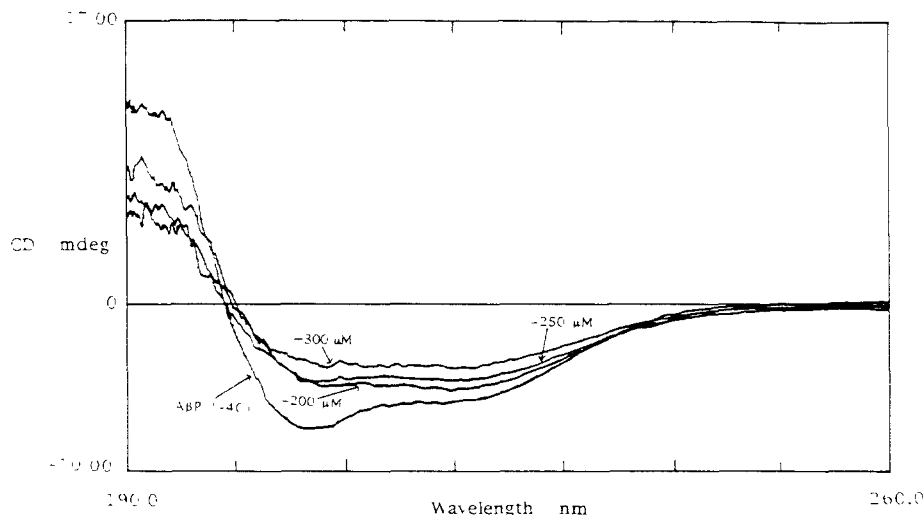

Fig. 1. The effect of aluminium on the CD spectrum of the AβP(1–40) polypeptide Spectra for 10, 20 and 50 μmol·dm⁻³ aluminium were identical to the pure peptide. The spectrum for 100 μmol dm⁻³ aluminium is not shown.

it has been suggested that this region of the polypeptide represents a possible nucleation site for acquisition of further β-sheet structure.

Addition of aluminium (50 μmol·dm⁻³) had a small but significant effect on the spectrum of AβP(25–35), with the principal changes observed at wavelengths below 210 nm (Fig. 2). Analyses based on the values of θ_{208} and on the CONTIN method indicated that the α-helical content had not changed, although there appeared to be a minor redistribution between β-sheet and remainder contents.

In marked contrast there was no detectable effect of this concentration of aluminium on the spectrum of AβP(1–40) (Fig. 1), presumably reflecting the greater conformational stability of the longer peptide. Consistent with this, there was no change in the intensity of tyrosine fluorescence of the AβP(1–40) peptide on addition of 50 μmol·dm⁻³ aluminium. Additions of higher concentrations of aluminium had a dramatic effect on the c.d. spectrum of AβP(1–40) (Fig. 1), the α-helical content of the peptide being all but abolished at an aluminium concentration of 300 μmol·dm⁻³ (Fig. 3). The effects of aluminium on the spectrum of this peptide were not uniform over the wavelength range and this may indicate that aluminium could cause a number of effects on the conformation by being bound at more than one site on the peptide. The increasing noise below 200 nm in the presence of the higher concentrations of aluminium made application of the CONTIN procedure less reliable; in general terms the analysis indicated a decrease in the α-helical and β-sheet contents and an increase in the remainder.

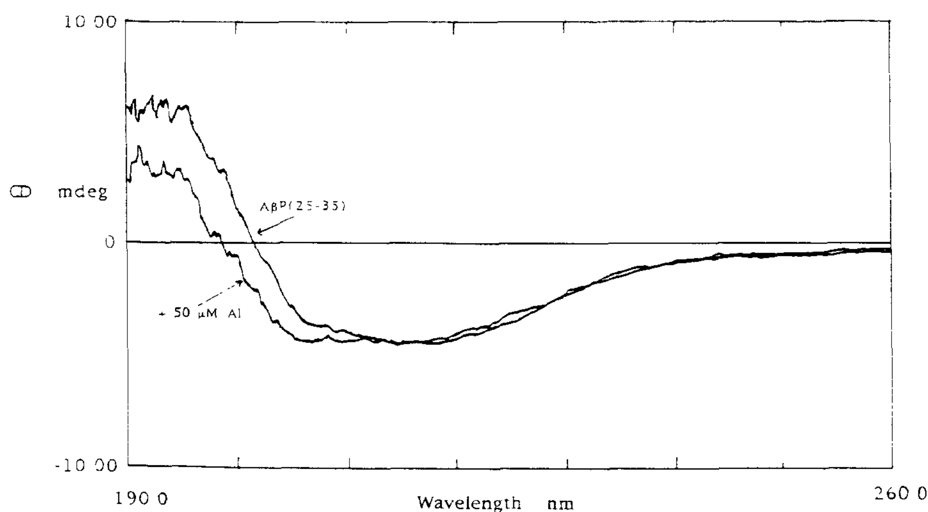

Fig. 2. The effect of aluminium on the CD spectrum of the AβP(25–35) polypeptide Spectra for 10 and 20 μmol·dm⁻³ aluminium were identical to the spectrum for the pure peptide

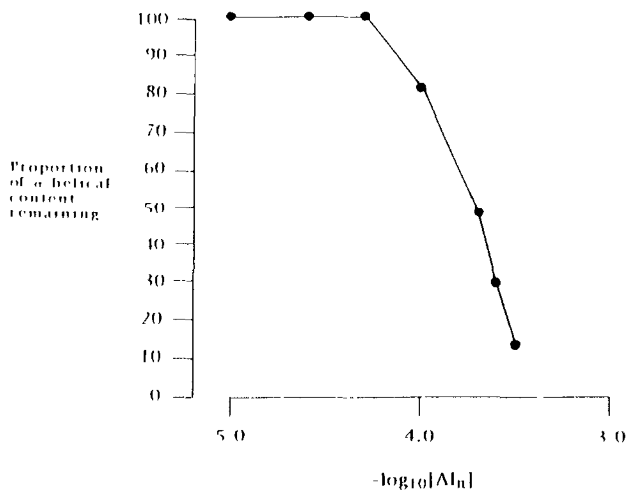

Fig. 3. The abolition by aluminium of the α-helical content of the AβP(1–40) polypeptide.

An in vivo association between aluminium and β-amyloid protein has been implied from various post-mortem analyses of the brains of AD sufferers [10]. The results presented herein represent the first evidence of an in vitro interaction of physiologically relevant concentrations of aluminium with the β-amyloid protein. The interactions are unique in terms of previous studies of aluminium and neuropeptides in that neurofilament phosphorylation [11] was not invoked as a prerequisite to an effect of aluminium. Our results support the recent demonstration that AβP(1–40) could act as a cation-selective transmembrane ion channel in vivo [5]. The blockade of this channel by aluminium might now be explained in terms of the capacity of aluminium to disrupt the α-helical conformation of the polypeptide.

How the interaction of aluminium with the β-amyloid protein might contribute towards the pathogenesis of Alzheimer's disease is not yet known. We have, however, given one of the first strong indications that the respective roles of aluminium (at concentrations below the sensitivity threshold of nuclear microscopy [12]) and β-amyloid in the aetiology of Alzheimer's disease may not be mutually exclusive.

Acknowledgements We thank SERC for the provision of the CD facility, and D.M. Mousdale for amino acid analyses. C.E acknowledges the support of ICI Watercare.

REFERENCES

[1] Jope, R S. and Johnson, G.V.W (1992) Ciba Found. Symp. 169, 254–267.
[2] Yankner, B A., Duffy, L.K. and Kirschner, D.A. (1990) Science 250, 279–282.
[3] Mattson, M.P., Cheng, B., Davis, D., Bryant, K., Lieberburg, I. and Rydel, R.E. (1992) J. Neurosci. 12, 376–389
[4] Exley, C. and Birchall, J.D. (1992) J. Theoret. Biol. 159, 83–98.
[5] Arispe, N., Rojas, E. and Pollard, H.B (1993) Proc. Natl. Acad. Sci. USA 90, 567–571
[6] Barrow, C.J., Yasuda, A., Kenny, P.T.M and Zagorski, M.G. (1992) J. Mol. Biol. 225, 1075–1093.
[7] Greenfield, N and Fasman, G.D. (1969) Biochemistry 8, 4108–4116.
[8] Provencher, S.W. and Glöckner, J. (1981) Biochemistry 20, 33–37.
[9] Woody, R W. (1974) in: Peptides, Polypeptides and Proteins. (Blout, E R., Bovey, F A., Goodman, M. and Lotan, M. eds.) pp. 338–350, Wiley, New York.
[10] Candy, J.M., Oakey, A.E., Klinowski, J., Carpenter, T.A., Perry, R.H., Atack, J.R., Perry, E.K., Blessed, G., Fairbairn, A. and Edwardson, J A. (1986) Lancet i, 354–357.
[11] Hollósi, M , Ürge, L., Perczel, A., Kajtár, Teplán, I., Otvös Jr., L and Fasman, G D. (1992) J. Mol. Biol. 223, 673–682.
[12] Landsberg, J.P., McDonald, B. and Watt, F. (1992) Nature 360, 65–68