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Amyloid β-peptide spin trapping I: peptide enzyme toxicity is related to free radical spin trap reactivitv

SYNTHETIC β-amyloid peptides (Aβs) demonstrate lot-to-lot variation in toxicity that has not been adequately explained. Studies from our laboratory have shown that Aβ toxicity may result from the ability of the peptide to promote oxidation reactions. Both Aβ(1–40) and Aβ(25–35) inactivate the oxidation-sensitive enzyme glutamine synthetase (GS) and generate electron paramagnetic resonance (EPR)-detectable products upon reaction with the spin trap phenyl-*tert*-butylnitrone (PBN). We now report the effect of synthetic Aβ(1–40) and Aβ(25–35) with attenuated toxicity with respect to peptide-induced GS inactivation, produce qualitatively different EPR spectra when the peptides are incubated with PBN. The results suggest an interpretation of conflicting observations regarding the toxicity of synthetic Aβs, and that investigators must be careful to assess the reactivity state of Aβ being studied.

Key words: Amyloid; Oxidation; Spin trapping; Phenyl-*tert*-butyl nitrone

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Introduction

β-Amyloid peptides (Aβs) are neurotoxic peptides postulated to be involved in the etiology of Alzheimer's disease (AD). The mechanism of neurotoxicity of Aβ [illegible] we proposed [illegible]

Anomalous Aβ samples, which demonstrated reduced toxicity toward GS, reacted with PBN to produce a four-line EPR spectrum. Furthermore, Aβ(25–35) samples which showed essentially no toxicity toward GS, generated no significant EPR signal upon incu-

Miller *et al*[8] and corrected for non-specific glutaminase activity by comparison of activity in the presence and absence of ADP and arsenate. GS enzyme (sheep brain, Sigma) and Aβ(25–35) were solubilized to a final concentration of 0.014 mg ml^{-1} and 1 mg ml^{-1}, respectively, and coincubated in deionized water for 1 h at 37°C

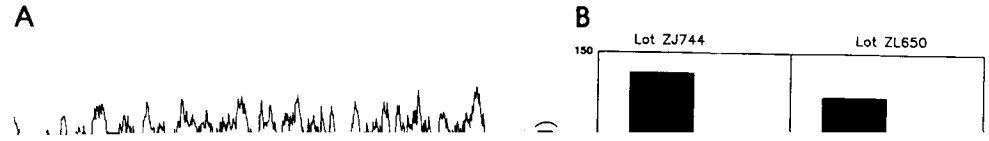
nate this reactive, highly toxic class of peptide Aβ(25–35)-A to distinguish it from four-line generating and EPR inactive (and correspondingly non-toxic) peptide variants discussed presently.

In the course of our investigations, we received shipments from Bachem of Aβ(25–35) which reacted with

2 mg ml^{-1} and 37°C for 24 h prior to GS addition.

HPLC/amino acid analysis: Amino acid analysis was performed on a Beckman 6300 HPLC amino acid analyzer utilizing orthophthalaldehyde (OPA) post-

intensities 1:2:2:1, Fig. 2B), in contrast to the three-line pattern routinely observed. For this spectrum, a_N = 14.5 G and ΔH = 0.9 G. This variation of Aβ(25–35) was found to be approximately half as toxic as the norm


A
B
Lot ZJ744
Lot ZL650
150

reactivity among variably toxic synthetic Aβ peptides.

Table 1. Amino acid analysis

		(dry)	(PBS)	(PBS/PBN)
GLY	3	2.57	2.81 ± 0.23	3.20 ± 0.21
SER	1	0.76	0.70 ± 0.03	0.58 ± 0.06
ASN	1	1.05	0.98 ± 0.03	0.90 ± 0.08
LYS	1	1.22	1.00 ± 0.13	0.81 ± 0.10
ALA	1	1.28	1.22 ± 0.04	1.27 ± 0.06
ILE	2	1.80	1.64 ± 0.08	1.91 ± 0.10
LEU	1	1.23	1.11 ± 0.06	1.36 ± 0.37
MET	1	0.90	0.62 ± 0.18	1.06 ± 0.08

Amino acid content (mol residue per mol peptide) in Aβ(25–35)-A dry

should perform potency assays to determine the reactivity state of individual Aβ samples prior to further experimentation. These observations also indicate that EPR spectroscopy offers such a means to ascertain the relative potency of Aβ peptides.

It is not yet clear whether the differences in Aβ reactivity stem from differences in the nature of the primary peptidyl radical center or, rather, from variable