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An antiserum to the extracellular domain of the Alzheimer amyloid precursor recognizes 70 and 88 kDa brain proteins

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An antiserum raised to the extracellular domain (residues 556–566) of the Alzheimer amyloid precursor protein recognized 70 and 88 kDa proteins in Western blots of rat, Alzheimer, Down's syndrome and control human brain separated by SDS-PAGE. The 70 kDa protein band was resolved into 5 spots by two-dimensional electrophoresis. The findings provide further evidence that a 70 kDa protein is a metabolite of the amyloid precursor protein and reveal an 88 kDa protein which was reduced in 3 out of 6 brains with Alzheimer pathology.

Amyloid precursor antiserum; Gel electrophoresis; Western blotting; Alzheimer-type dementia; Down's syndrome

1. INTRODUCTION

Amyloid, an aggregate of a 42–43 amino acid peptide [1], is deposited in brain plaques and blood vessels in ATD and Down's syndrome. A search for possible metabolites of the amyloid precursor protein (proA4) revealed a soluble protein of approximately 70 kDa in human and rat brain which was recognized by an antiserum raised to the C-terminus of proA4 (anti-C) [2]. In this study, we provide further evidence that the 70 kDa protein is related to proA4 and describe an 88 kDa protein also recognized by an antiserum raised to the putative extracellular domain of proA4 (anti-E).

2. EXPERIMENTAL

2.1. *Production of antiserum to the extracellular domain of proA4*

The peptide H_2N-CANTENEVEPVD-COOH, consisting of a region just extracellular to the transmembrane domain of proA4 (residues 556–566 in [1]) with an N-terminal cysteine to permit conjugation to thyroglobulin, was synthesized and an antiserum (anti-E) raised in rabbits as described previously [2].

2.2. *Preparation of tissue samples for SDS-PAGE*

Frozen human autopsy tissues and fresh rat brain, cerebral cortex and hypothalamus were prepared with slight modifications to the methods described previously [2]. Tissue homogenates in Tris buffer were centrifuged at 14000 × *g* for 18 min, and the supernatant and pellet fractions prepared for SDS-PAGE by boiling for 5 min in the presence of 2% SDS and 5% 2-mercaptoethanol. A supernatant fraction from rat PC12 pheochromocytoma cells was similarly prepared following incubation of the cells for 30 min at 0°C with 2% Triton X-100 and centrifugation at 14000 × *g* for 10 min.

2.3. *SDS-PAGE, Western blotting and immunostaining*

Samples containing 2.05–3.40 μg protein were applied to 10–15% micro-gradient gels and SDS-PAGE performed on a PhastSystem apparatus (Pharmacia Ltd). The separated proteins were transferred to polyvinylidene difluoride membranes (Immobilon, Millipore Ltd) by diffusion at 70°C on the PhastSystem. Immunostaining of blotted proteins with anti-E and anti-C was performed [2] with 1:1200 (fig.1a,b) or 1:2000 (fig.1c–o, fig.2) dilutions of the antisera. The specificity of staining was examined with antiserum containing antigen peptide (300 μg/ml antiserum).

2.4. *2-D gel electrophoresis*

A supernatant fraction prepared in 1 mM EDTA, 10 mM Tris-HCl, pH 7.5, from frontal cortex of a control case (65 years) was diluted in buffer containing 9.5 M urea and 2% Nonidet P40, and 50 μg sample protein subjected to 2-D electrophoresis using a modified O'Farrell technique [3]. The first-dimension gel contained 2% Pharmalytes (pH 4–6.5:pH 3–10, 4:1; Pharmacia Ltd). The second dimension was run on precast gradient minigels (type PAA 4/30, Pharmacia Ltd) and was followed by electrophoretic transfer [2] of the separated proteins to Immobilon.

3. RESULTS

The antiserum raised to the extracellular domain of proA4 (anti-E) bound to several proteins on Western blots of human cerebral cortex separated by SDS-PAGE. Only two of these proteins (approximately 70 and 88 kDa) were stained specifically in that they were not stained by antiserum blocked by preincubation with the antigen peptide (fig.1a,b). The 70 kDa protein was

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Abbreviations: Anti-E (anti-C), antiserum to the extracellular domain (C-terminus) of ProA4; ATD, Alzheimer-type dementia; ProA4, amyloid precursor protein; SDS-PAGE, SDS-polyacrylamide gel electrophoresis

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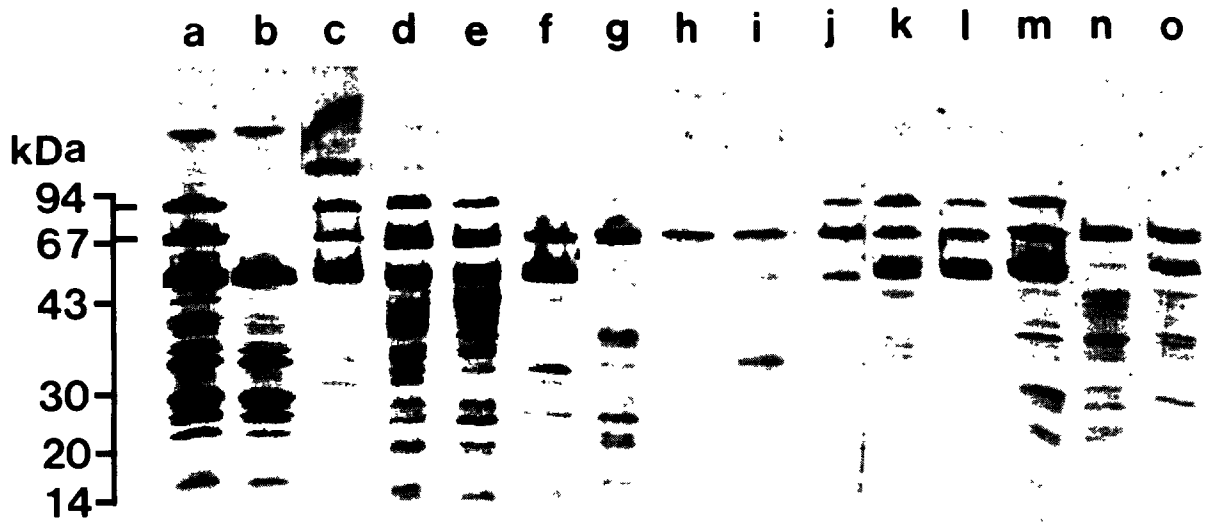

Fig.1. Western blots of various tissue samples separated by SDS-PAGE and immunostained with anti-E. All samples were 14000 × *g* supernatants of tissue homogenates, as in section 2.2, except for (f) and (j). (a) Human frontal cortex; (b) as (a) but with blocked antiserum; (c) rat midbrain; (d) human cerebellum; (e) human corpus callosum; (f) human temporal cortex: particulate fraction; (g) human liver; (h) human kidney; (i) human skeletal muscle; (j) PC12 cells: Triton X-100 soluble fraction; (k) ATD temporal cortex: 80 years; (l) control temporal cortex: 80 years; (m) Down's temporal cortex: 1.5 years; (n) Down's temporal cortex: 63 years; (o) ATD temporal cortex: 88 years.

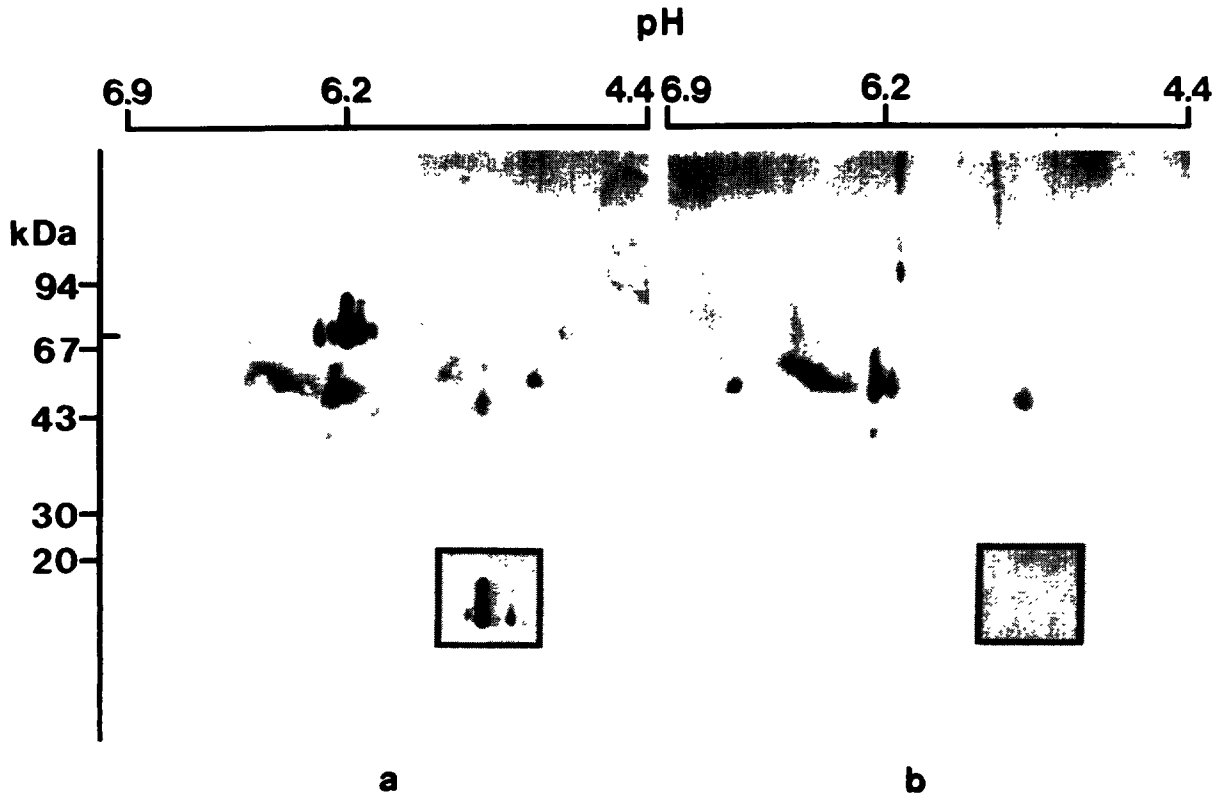

Fig.2. 2-D blot of human frontal cortex immunostained with anti-C and (insert) anti-E. (a) Unblocked antisera; (b) antisera blocked with the appropriate proA4 peptide. The major spots in (a) and the insert are identical.

occasionally resolved into a closely-spaced doublet band when stained with anti-E (e.g. fig.1n), whereas a single band was always observed with anti-C [2].

When visualized with anti-C, the 70 kDa protein was confined largely to the Tris buffer-soluble fraction of brain tissue, and little staining was observed in the soluble or particulate fractions of peripheral tissues [2]. A similar distribution was observed for the 70 and 88 kDa proteins visualised with anti-E. The supernatant fractions of rat cerebral cortex, hypothalamus and midbrain, and human frontal cortex, temporal cortex, cerebellum and corpus callosum showed marked immunoreactivity, whereas the SDS-extracted particulate fractions showed weak immunoreactivity (fig.1a,c–f,l). Human liver, kidney and skeletal muscle contained a little 70 kDa protein but no detectable 88 kDa protein (fig.1g–i). An extract of cultured rat PC12 cells contained 70 and 88 kDa proteins (fig.1j).

Blots of temporal cortex from 6 controls (1.2 years and 65–82 years) and 2 young cases of Down's syndrome (1.5 and 27 years) stained with anti-E showed similar 70 and 88 kDa immunoreactivity (fig.1l,m). Comparison of blots from 5 ATD cases (65–88 years) and one Down's case with brain plaques and tangles (62 years) with blots from the 5 elderly controls showed no difference in the 70 kDa protein but a reduced staining of the 88 kDa protein in 2 ATD cases (65 and 88 years) and the Down's case (fig.1k,l,n,o).

In blots of 2-D gels of human cerebral cortex incubated with anti-E, the 70 kDa staining was resolved into 2 spots in the p*I* 6.2 region (measured with a surface microelectrode) but the 88 kDa protein was not observed. With anti-C, the staining at 70 kDa could be resolved into 5 spots (fig.2). These spots appeared to represent relatively abundant tissue proteins as judged by Coomassie blue staining.

4. DISCUSSION

Previously [2], we suggested that the 70 kDa brain protein recognized by anti-C, our antiserum to the C-terminus of proA4, could be a proA4-related protein or a protein unrelated to the amyloid precursor but containing an epitope found in proA4. The present results make it much more likely that the 70 kDa protein is indeed related to proA4 since a 70 kDa protein was also recognized by an antiserum (anti-E) raised to another region of proA4. The 70 kDa protein band stained by anti-C in SDS-PAGE gels consists of at least 5 proteins with slightly different p*I*s, two of which are recognized by anti-E. These proteins may arise through post-translational modifications (e.g. glycosylation, phosphorylation) of a single proA4-related protein. The 88 kDa protein recognized by anti-E was reduced in some cases with Alzheimer pathology but did not appear in 2-D gels, possibly because its p*I* was outside the range of our gel system or because its antibody binding site was sensitive to the conditions used in the first dimension.

Other workers [4,5] have confirmed our finding [2] that a soluble brain protein of approximately 70 kDa can be detected with antisera to the C-terminal pentadecapeptide of proA4, and Kametani et al. [4] suggest that this protein may be the final intermediate in the process of amyloid formation. By contrast, Weidemann et al. [6] suggest that proA4 is cleaved to an extracellular N-terminal 91 kDa protein (which may resemble our 88 kDa protein) and a transmembrane 17–18 kDa C-terminal protein from which amyloid is formed. It is possible that more than one metabolic pathway is involved in the processing of proA4, and an imbalance between pathways may lead to amyloid deposition.

In conclusion, we have identified 2 proteins of 70 and 88 kDa which are probable metabolites of the amyloid precursor protein, and which may be involved in amyloid deposition in ATD and Down's syndrome. Resolution of these proteins by 2-D gels and the use of (PC12) cells in culture are valuable tools which may allow elucidation of the metabolism of proA4 and ultimately the mechanism of amyloid deposition.

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REFERENCES

[1] Kang, J., Lemaire, H.-G., Unterbeck, A., Salbaum, J.M., Masters, C.L., Grzeschik, K.-H., Multhaup, G., Beyreuther, K. and Müller-Hill, B. (1987) Nature 325, 733–736.
[2] Simpson, J., Bladon, C.M., Yates, C.M. and Harmar, A.J. (1988) FEBS Lett. 237, 196–198.
[3] O'Farrell, P.H. (1974) J. Biol. Chem. 250, 4007–4021.
[4] Kametani, F., Tanaka, K., Haga, S., Satoh, M., Allsop, D. and Ishii, T. (1989) Biomed. Res. 10, 179–183.
[5] Selkoe, D.J., Berman Podlisny, M., Joachim, C.L., Vickers, E.A., Lee, G., Fritz, L.C. and Oltersdorf, T. (1988) Proc. Natl. Acad. Sci. USA 85, 7341–7345.
[6] Weidemann, A., König, G., Bunke, D., Fischer, P., Salbaum, J.M., Masters, C.L. and Beyreuther, K. (1989) Cell 57, 115–126.