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Cross-linking of a synthetic partial-length (1–28) peptide of the Alzheimer β/A4 amyloid protein by transglutaminase

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Cerebral deposits of β/A4 amyloid protein is a pathologic sign of Alzheimer's disease. A synthetic partial-length (1–28) peptide of this protein contains one glutamine and two lysine residues. Here we show that this peptide can be a substrate of transglutaminase, which catalyzes cross-linking between glutamine and lysine residues in peptides, by demonstrating the formation of multimeric peptides due to the action of this enzyme. A modified (Lys^{28} to L-norleucine) version of the synthetic peptide was also cross-linked, but another modified version (Lys^{16} to L-norleucine) was very poorly cross-linked, indicating that Lys^{16} is involved exclusively in the cross-linking of the partial-length peptide catalyzed by transglutaminase.

Transglutaminase; Amyloid beta-protein; Alzheimer's disease.

1. INTRODUCTION

Alzheimer's disease is characterized by extracellular deposits in the brain of insoluble aggregates of amyloid proteins [1]. The main component of the deposits is a 4-kDa β/A4 amyloid protein [2,3]. This protein arises from proteolytic cleavage of one or more large transmembrane proteins, the β/A4 amyloid protein precursor(s) [4–6]. The cerebral deposits of β/A4 amyloid protein seem to contribute to the pathogenesis of Alzheimer's disease [7]. The mechanism of extracellular accumulation of this protein is unknown. Amyloid deposits consist of fibers 4 to 10 nm in diameter. The β/A4 amyloid protein in amyloid fibrils is organized into a cross-β conformation in which the peptide backbone is perpendicular to the fiber axis [8]. Studies using synthetic peptides containing partial- and full-length sequences of β/A4 amyloid protein have suggested that the physical properties of β/A4 amyloid protein themselves are the causes of amyloid fibril assembly and the formation of insoluble aggregates [9–14].

Transglutaminases (protein-glutamine:amine γ-glutamyltransferase, EC 2.3.2.13) are Ca^{2+}-dependent enzymes that catalyze the formation of ε-(γ-glutamyl)lysine cross-links between glutamine and lysine residues of certain proteins and are involved in several biological functions (for review, see [15–17]). These functions include blood clotting, wound healing, keratinization of the epidermis, and stiffening of erythrocyte membranes, in which substrate proteins are cross-linked into polymers of high molecular weight that are insoluble in denaturants and resistant to proteinases. Selkoe et al. [18,19] have characterized the transglutaminase activity in the human brain and suggested that transglutaminase is involved in the intracellular formation of helically wound intermediate filaments that make up the neurofibrillary tangles found in Alzheimer's disease. The possibility that transglutaminase is involved in the extracellular formation of the insoluble amyloid deposits has not been examined. Here we report intermolecular cross-linking of a synthetic partial-length (1–28) peptide of the Alzheimer β/A4 amyloid protein by transglutaminase in vitro and suggest that this enzyme is involved in the formation of the deposits.

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2. MATERIALS AND METHODS

The following materials were obtained from the sources indicated: partial-length (1–28) peptide of β/A4 amyloid protein (Bachem); modified partial-length (1–28) peptides of β/A4 amyloid protein (K16nL and K28nL) in which Lys^{16} or Lys^{28} was replaced by L-norleucine (Iwaki Glass); gel plates for gradient SDS-PAGE (Daiichi Pure Chemicals); and molecular mass marker proteins, LMW (Pharmacia). Transglutaminase was purified from a homogenate of guinea pig liver on an immunoadsorbent column as described previously [20]. The reaction mixture for the cross-linking of peptides contained 40 mM Tris-HCl, pH 7.5, 5 mM $CaCl_2$, 10 mM dithiothreitol, 50 μg/ml transglutaminase, and 0.9 mg/ml peptide as the substrate. The reaction took place at 37°C and was ended by the addition of a 1/10 volume of 0.4 M EDTA. Before SDS-PAGE, control peptides dissolved in 10 mM Tris-HCl, pH 7.5, and mixtures in which the reaction had been stopped were mixed with an equal volume of 2 × sample buffer by the method of Laemmli [21] and immersed in boiling water for 1.5 min. SDS-PAGE was done with an SDS-polyacrylamide gradient (15 to 20%) gel and electrode buffer as in the method of Laemmli. The gel

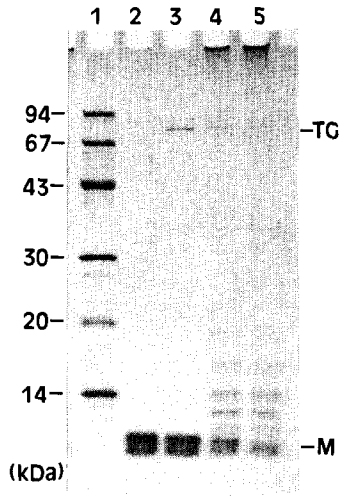

Fig. 1. SDS-PAGE pattern of partial-length (1–28) peptide during transglutaminase treatment. Lane 1, molecular mass marker proteins (from top, phosphorylase b, bovine serum albumin, ovalbumin, carbonic anhydrase, soybean trypsin inhibitor, and α-lactoglobulin; numbers on the left show the molecular masses of these marker proteins). Lanes 2 to 5 each contained 3.6 μg of the peptide. Lane 2, partial-length (1–28) peptide; lane 3, peptide incubated with transglutaminase for 180 min in the presence of 36 mM EDTA; lanes 4 and 5, peptide treated with transglutaminase for 20 and 180 min, respectively. 'M' indicates the position of the monomer peptide band and 'TG' indicates the position of the transglutaminase band.

was stained with a solution containing 0.2% Coomassie brilliant blue, 40% methanol, and 10% acetic acid and destained in 7% acetic acid.

3. RESULTS AND DISCUSSION

The partial-length peptide (1–28; DAEFRHDSGYEVHHQKLVFFAEDVGSNK) of the Alzheimer β/A4 amyloid protein contains a glutamine residue at position 15 and lysine residues at positions 16 and 28. For detection of intermolecular cross-links formed by transglutaminase between partial-length peptides, changes in the molecular size of peptides incubated with transglutaminase were analyzed by gradient (15–25%) SDS-PAGE (Fig. 1). Incubation for 20 or 180 min caused the disappearance of more than half of the monomer peptide, and new bands with slower electrophoretic mobilities appropriate for multimer peptides appeared (lanes 4 and 5). Animal transglutaminases need calcium ions as a cofactor. In the presence of EDTA, multimer peptides were not produced, and the monomer peptide remained unchanged (lane 3). These results indicate that cross-linking between the peptides was caused by transglutaminase. A few components of high molecular weight that did not move through the gel were detected in the products of 20- and 180-min reactions (lanes 4 and 5). These components seemed to be polymerized transglutaminase, because the monomeric transglutaminase band became fainter as the incubation time increased. Further studies are needed to determine whether the peptide is present in the high molecular weight components.

Multimer peptides are produced probably by the formation of an intermolecular isopeptide bond between Gln^{15} and either Lys^{16} or Lys^{28}. For identification of which lysine residue was involved in the cross-linking catalyzed by transglutaminase, two modified partial-length peptides, K16nL and K28nL, were compared for their efficiency as substrates for transglutaminase; Lys^{16} or Lys^{28}, respectively, was replaced by L-norleucine. The cross-linked products of the modified peptides were analyzed by SDS-PAGE (Fig. 2). The K28nL peptide (lanes 10 and 11) was cross-linked to multimers with a reactivity similar to that of the original partial-length peptide (lane 3), but the K16nL peptide (lanes 6 and 7) was a poor substrate. Lys^{16} more often participated in the transglutaminase-catalyzed cross-linking of the partial-length peptide. The reaction products from K16nL (lanes 6 and 7) and K28nL (lanes 10 and 11) peptides gave a band with faster mobility than that of the monomer band, but the reason for the appearance of the new band with faster mobility is not known.

Transglutaminase and β/A4 amyloid protein are likely to be present together in extracellular sites in the brain, because transglutaminase activity has been detected on the outside surface of rat brain synaptosomes [22]. For more evidence of our suggestion that transglutaminase is involved in the formation of the insoluble amyloid deposits found in Alzheimer's disease, it would be necessary to show that there is an ε-(γ-glutamyl)lysine isopeptide bond in the deposits. The β/A4

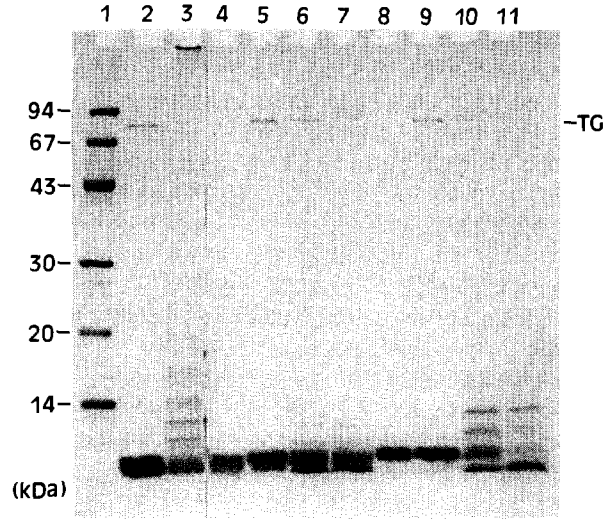

Fig. 2. SDS-PAGE pattern of modified partial-length peptides during transglutaminase treatment. Lane 1, molecular mass marker proteins (see legend of Fig. 1). Lanes 2 to 11 each contained 3.6 μg of the peptide. Lanes 2 and 3, partial-length (1–28) peptide treated with transglutaminase for 0 and 180 min, respectively; lane 4, K16nL peptide; lanes 5–7, K16nL peptide treated with transglutaminase for 0, 20, and 180 min, respectively; lane 8, K28nL peptide; lanes 9–11, K28nL peptide treated with transglutaminase for 0, 20, and 180 min, respectively. For 'M' and 'TG', see the legend of Fig. 1.

protein is produced in soluble form both in vitro and in vivo during normal cellular metabolism [23–25]. For understanding of the pathological processes in Alzheimer's disease, the mechanism by which the soluble β/A4 protein is converted to the insoluble form must be identified.

In hereditary cerebral hemorrhage with amyloidosis of the Dutch type, subjects develop deposits of β/A4 amyloid protein around meningeal and cerebral blood vessels. Two patients with this disease were found to have a point mutation resulting in the replacement of glutamic acid by glutamine at position 22 of the β/A4 amyloid protein [26]. This mutation may make the β/A4 protein a better substrate for transglutaminase by increasing the number of glutamine residues, leading to intermolecular cross-linking that facilitates the formation of amyloid deposits.

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