Molecular Structures and High Resolution TEM

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Electron microscopic visualization of dipeptidyl peptidase IV influence on the formation of nano-fibrils of amyloid beta peptide

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The aggregates of amyloid beta peptides (Aβs) are considered as one of the main pathological hallmarks of Alzheimer's disease (AD). Formation of Aβs depends on processing of Amyloid Precursor Protein (APP) by β- and γ-secretases[1-3]. Misprocessing of APP leads to a rise of Aβs including 39-42 amino acids whose amyloid fibril forms constitute a primary component of amyloid plaques found in the brains of AD patients. In the present work, we demonstrated for the first time the principal ability of dipeptidyl peptidase IV (DPPIV) to cleave the commercial Aβ40 and Aβ42 *in vitro*, to influence on their fibril formation property and to participate in the disaggregation of preformed fibrils. In the visualization processes, transmission electron microscope (TEM) was employed, using grid staining (negative staining with UAc, PTA, STA). Measurements of the fibril dimensions were taken on enlarged prints of electron micrographs. In the experiments, Aβ40 and Aβ42 from Sigma, Abbiotec and Bachem were used. We monitored the fibrillation of the peptides both in the absence and in the presence of DPPIV. In Figure 1, A, the transmission electron microscope visualization of fibrils, formed during incubation of 135 μM Aβ40 during 3 days in the phosphate buffer, pH 7.4, is presented. The electron-microscopic measurements were fulfilled on the transmission electronic microscopes of Tesla and Tescan firms, provided with the microanalysis devices. The negative contrast staining of the sample with 2% phosphate-tungsten solution, pH 7.0 was used. To study the influence of DPPIV on the fibrillation process, the same experiment was performed with the addition of 1.3 μM DPPIV. The result of visualization of this sample is presented in Figure 1, B. It demonstrates that the presence of the enzyme in the incubation mixture essentially hindered the fibrillation of the peptide. The possible influence of DPPIV on the preformed fibrils of Aβ40, a sample identical to that, shown in Figure 1, A, was incubated during the next 3 days in the presence of 1.6 μM DPPIV. The result of this incubation (Figure 1, C) manifests that the preformed fibrils are markedly broken down to shorter fibrils with an apparent diameter 8-25 nm as a result of DPPIV including into the assay mixture. The similar results were received with the amyloid peptide Aβ42. In conclusion: the electron microscopic pictures in Figure 1 A, B and C, prove the suggestion regarding the ability of DPPIV to hinder the aggregation of amyloid peptides and to disaggregate the preformed fibrils.

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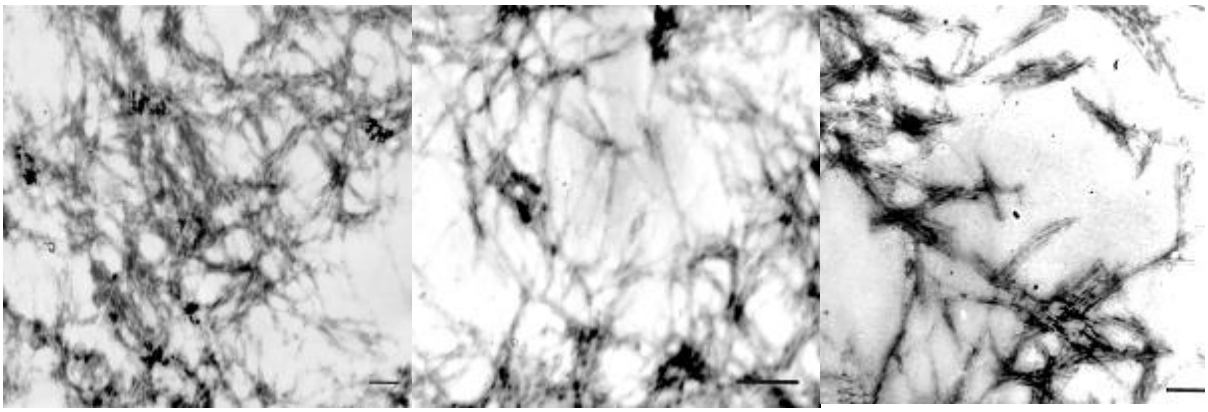

a.) b.) c.)

Figure 1. The transmission electron microscopy visualization of 135 μM Aβ40 (Bachem) fibrils (negative staining) formed during incubation during 3 days of the peptide alone (A); in the presence of 1.3 μM DPPIV (B); the same as in (A), after additional 3 days' incubation with 1.6 μM DPPIV (C). Scale bar: 100 nm