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BREAKDOWN OF SOME NEURONAL PEPTIDES WITH DIPEPTIDYL PEPTIDASE IV

N. M. MOVSISYAN, S. G. SHAROYAN, A. A. ANTONYAN, S. S. MARDANYAN*

H. Buniatyan Institute of Biochemistry of NAS RA, Armenia

It is shown that the amyloid beta peptides Aβ(1-16) and Aβ(1-40) and a cytokine from neurosecretory granules of bovine neurohypophysis, Proline Rich Peptide PRP-4, are truncated at the presence of multifunctional dipeptidyl peptidase IV (CD26/DPPIV), widely expressed in different mammalian tissues. It means that these neuropeptides represent substrates of the enzyme. Besides, it appeared that at the presence of CD26/DPPIV the process of Aβ(1-40) aggregation *in vitro* was hindered.

***Keywords*:** amyloid beta peptides, dipeptidyl peptidase IV, fibril formation, Proline Rich Peptide 4, enzymatic cleavage of Aβ peptides.

Introduction. The aggregates of amyloid beta peptides (Aβs) are considered as one of the main pathological hallmarks of Alzheimer's disease and related disorders [1, 2]. Misfolding and aggregation of these peptides are largely responsible for their neurotoxicity [3]. It was demonstrated [4] that the neurotoxicity of Aβs strongly correlates with their aggregative ability. It is expected that Aβ proteolysis can lead to the decrease in its steady-state concentration and deposition in brain.

On the other hand, proteolytic processing of proline-containing neuropeptides and hormones from precursors, and their breakdown is realized by proteases specific for proline bonds. Dipeptidyl peptidases (DPPs) compose a family of proteases removing dipeptides from N-terminus of peptides with penultimate Pro and Ala [5]. DPPIV (E.C. 3.4.14.5) is an enzyme of this family [6]. In the hematopoiesis system DPPIV is expressed on the surface of T-cells as antigen CD26, and CD26/DPPIV participates in immune response formation, activation of T-cells, cell adhesion, modulation of proline-containing peptides [7], etc. To elucidate the possible influence of CD26/DPPIV, widely expressed in blood cells, on the fate of proline rich peptides in the mammalian blood, earlier, we have shown that the Proline Rich Peptide-1 (Ala-Gly-Ala-Pro-Glu-Pro-Ala-Glu-Pro-Ala-Gln--Pro-Gly-Val-Tyr), discovered by academician Galoyan with colleagues [8], is a substrate for DPPIV [9].

The goal of the present work was to investigate the ability of CD26/DPPIV, purified from bovine kidney, to truncate the commercial Aβ(1-16) and Aβ(1-40) peptides and to influence their fibril formation property, as well as to assay, if the

* E-mail: biochem@ipia.sci.am

other Proline Rich Peptide from neurosecretory granules of neurohypophysis, PRP-4 (Ala-Pro-Glu-Pro-Ala-Glu-Pro-Ala-Gln-Pro), might also be degraded by CD26/DPPIV.

Experimental Part. The electrophoretically homogenous preparation of DPPIV with the specific activity 10 *μmol* of synthetic substrate (Gly-Pro-pNA) cleaving per *min* per *mg* of protein was purified as described earlier [10]. Aβ(1-16), Aβ(1-40), Gly-Pro-pNA, 2-mercaptoethanol, Thioflavin-T (ThT), ovalbumin, BSA, etc. were purchased from "Sigma". Only the highest available purity reagents were used.

The method of controlling peptide cutting in the presence of purified DPPIV was based on the estimation of the amount of primary amines increasing during incubation of the peptide in the presence of DPPIV. The quantity of primary amines was evaluated using o-phtalaldehyde (OPA) dye, which at the presence of 2-mercaptoethanol forms with the primary amines a chromophore characterized by absorption maximum at 334 *nm* [11].

The formation of Aβ(1-40) fibrils proceeded in 3 days' incubation at 37^0C of 100–150 *μM* protein solution in 20 *mM* phosphate buffer, pH 7.4. The same conditions were used for studying the influence of 1.2 *μM* DPPIV on this process. To ensure, that this influence is DPPIV-specific, identical experiments were performed replacing DPPIV with 1.2 *μM* ovalbumin or BSA.

For monitoring the formation of Aβ(1-40) fibrils/aggregates, fluorimetric measurements were used. Thioflavin-T (ThT) associates rapidly with fibril and aggregated forms of peptides resulting the appearing of fluorescence with λ_{ex}= 420 *nm* and λ_{em}= 487 *nm* [12, 13]. 2 *mM* stock solution of ThT in water was used after dilution to 100 *μM*. The assay mixture for fluorescence registration in 240 *μl* of 10 *mM* Phosphate buffer, pH 7.4 contained: 0.1 *mM* KCl, 8 *μM* ThT and 10 *μl* of sample.

Spectral measurements were performed on Specord M-40 UV-VIS spectrophotometer (Germany); fluorimetric measurements – on spectrofluorimeter Perkin-Elmer MPF-44A (USA), in quartz cuvettes, light path 0.5–1.0 *cm*, at 25°*C* using thermostated cuvette holders.

Results and Discussion.

1. *Breakdown of Amyloid beta peptides with Dipeptidyl Peptidase IV.* Impaired clearance of Amyloid β peptides (Aβs) from the brain likely contributes to the Alzheimer's disease. Decreased proteolysis of Aβs would lead to an increase in the steady-state concentration of peptides and a subsequent increase in their deposition. Several major pathways for Aβs clearance exist, one of them – direct proteolytic degradation. A number of peptidases participate in cleaving of Aβs *in vitro* [14].

Considering the presence of N-terminal penultimate alanine in Aβ peptides: Asp-Ala-Glu-Phe-Arg-His-Asp-Ser-Gly-Tyr-Glu-Val-His-His-Gln-Lys-Leu-Val--Phe-Phe-Ala-Glu-Asp-Val-Gly-Ser-Asn-Lys-Gly-Ala-Ile-Ile-Gly-Leu-Met-Val-Gly--Gly-Val-Val-Ile-Ala, we presumed that DPPIV could be one of the proteases, contributing to the cleavage of Aβs.

In our *in vitro* investigations we have shown, that DPPIV was able to truncate the commercial Aβ(1-16) and Aβ(1-40) peptides (Fig. 1, A and B).

Absorbance at 334 *nm* (due to the formation of the complex between primary amines with an orto-phtalaldehide) reflexes the number of primary amines, which increases because of the truncation of peptide links during incubation.

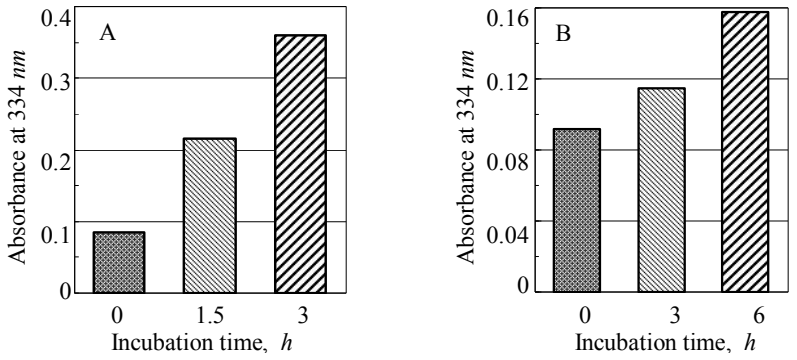

Fig. 1. Truncation of Aβ(1-16) (A) and of Aβ(1-40) (B) by DPPIV.

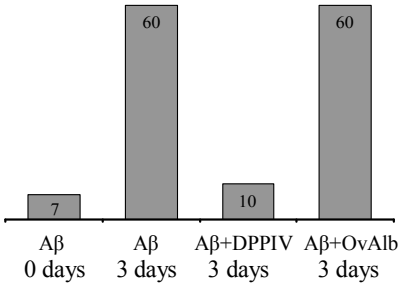

Fig. 2. Intensity of Thioflavin-T fluorescence (λ_{ex}=420 *nm*, λ_{em}=482 *nm*) of a fresh solution of Aβ(1-40) and the identical solutions after incubation during 3 days with and without DPPIV and ovalbumin.

2. *Influence of DPPIV on the aggregation of Aβ(1-40).* As the truncation of Aβ peptides may influence on their aggregation properties, we monitored the process of Aβ(1-40) aggregation using Thioflavin-T fluorescence, which is known as reflecting the aggregation state of amyloid peptides [12]. In Fig. 2 the intensity of Thioflavin-T fluorescence of fresh solution of Aβ(1-40) and the identical solutions after incubation during 3 days with and without DPPIV and ovalbumin (as a negative control) are shown. The results of this experiment indicated that the aggregation/fibrillation of Aβ(1-40) peptide was hindered in the presence of DPPIV.

3. *Breakdown of PRP-4 with DPPIV.* In the preliminary experiment the increase of amount of primary amines in the aliquots of the assay mixture at the beginning and after 24 hour incubation of PRP-4 at the presence of soluble DPPIV evidenced the truncation of this cytokine.

The time-dependent increase of amount of primary amines in the assay mixture was measured using OPA. PRP-4 was incubated during 1.5 *h* at room temperature at the presence of DPPIV. The observed increasing of absorbance at 334 *nm* due to NH_2-OPA complex formation (Fig. 3) evidenced the truncation of peptide links in PRP-4 by the enzyme.

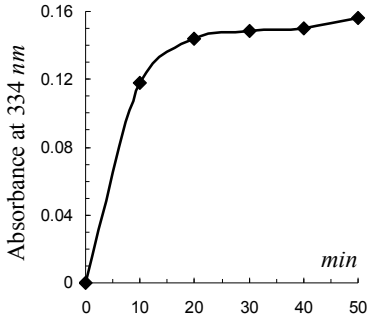

Fig. 3. The time-dependent increase of amount of primary amines upon incubation of PRP-4 at the presence of DPPIV.

4. *Parameters of enzymatic reaction of PRP-4 breakdown by DPPIV.* To determine the parameters of enzymatic reaction of PRP-4 breakdown catalyzed by DPPIV, the dependence of increase of optical absorbance at 334 *nm* due to cutting off the peptide links was measured in the assay mixture containing enzyme and different concentrations of cytokine. The graphical analysis based on the double-reciprocal dependence of number of primary amines and PRP-4 concentration

(Lineweaver-Burk plots) allowed to evaluate the parameters of DPPIV-catalyzed enzymatic reaction as: V_{max}= 3.1± 0.2 *nmol*/*min* and K_m= 0.114±0.01 *mM*.

Conclusions.

1. It is shown that a cytokine from neurosecretory granules of bovine neurohypophysis, Proline Rich Neuropeptide PRP-4, possessing a lot of physiological functions, is degraded by the widely expressed in different mammalian tissues multifunctional DPPIV and represents a new natural substrate of the enzyme.

2. Amyloid Aβ(1-16) and Aβ(1-40) peptides represent new substrates of DPPIV and are degraded by the enzyme *in vitro*.

3. At the presence of DPPIV the process of Aβ(1-40) aggregation *in vitro* is hindered.

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