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SCIENTIFIC REVIEWS

Prolyl Endopeptidase Enzyme Inhibitors of Plant Origin

İlkay ORHAN*°, Gürdal ORHAN**, Bilge ŞENER*

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Summary : *Prolyl endopeptidase (PEP) is a serine protease which is known to play a role in degradation of proline-containing neuropeptides involved in the processes of learning and memory. PEP inhibitors are expected to exert their beneficial effects by increasing the brain levels of those neuropeptides which may improve and restore cognitive functions and protect vulnerable nerves against damage and cell death. Therefore, they are considered to have therapeutic potential against Alzheimer's Disease. To date many compounds have been isolated from natural sources including plants, fungi and bacteria. Their synthetic counterparts have been also studied. This review covers PEP inhibitors isolated from plants.*

Key Words: *Prolyl endopeptidase, Alzheimer's disease, neuropeptide, PEP inhibitory activity, plant*

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Bitkisel Kaynaklı Prolil Endopeptidaz Enzim İnhibitörleri

Özet : *Prolil endopeptidaz (PEP), öğrenme ve hafıza sürecinde etkili prolin içerikli nöropeptitlerin yıkılmasında rol oynadığı bilinen bir serin proteazdır. PEP inhibitörlerinin sinirleri hasar ve hücre ölümüne karşı koruyan, tanıma fonksiyonlarını yeniden yapılandırıp iyileştirebilen nöropeptitlerin beyin düzeylerini artırarak yararlı olmaları beklenir. Bugüne kadar, sentetik eşdeğerleri olduğu kadar, Alzheimer hastalığına karşı terapötik bir potansiyele sahip PEP inhibitör aktiviteli pek çok bileşik, bitkiler, mantarlar ve bakteriler de dahil olmak üzere doğal kaynaklardan izole edilmiştir. Bu derleme, bitkilerden elde edilen PEP inhibitörlerini kapsamaktadır.*

Anahtar kelimeler : *Prolil endopeptidaz, Alzheimer hastalığı, nöropeptit, PEP inhibitör aktivite, bitki*

INTRODUCTION

Prolyl endopeptidase (PEP, also called prolyl oligopeptidase) is a cytosolic endopeptidase involved in the degradation of several proline-containing neuropeptides implicated in learning and memory processes. These neuropeptides such as vasopressin, substance P, thyrotropin-releasing hormone (TRH), oxytocin, neurotensin, angiotensins and opioids are affected in Parkinson Disease. Proline endopeptidase (PEP) contributes to the degradation of many of these neuropeptides, some of which are linked to a variety of cognitive functions[1,2] and could also control the production of the amyloidogenic peptide A-beta.

There is also some evidence that psychiatric disorders, such as major depression, schizophrenia and post-traumatic stress disorder are associated with significant alterations in the activity of some enzymes including prolyl endopeptidase (PEP), acetylcholinesterase (AChE) and dipeptidyl peptidase-IV (DPP-IV). Therefore, potent, selective and permanent inhibitors of PEP could serve as probes to assess the genuine contribution of this enzyme in Alzheimer's pathology[3-5]

Formation of beta-amyloid and neurofibrillary tangles in the brain due to genetic or other factors and marked reduction of certain brain neuropeptide lev-

* Gazi University, Department of Pharmacognosy, Faculty of Pharmacy, 0633 Ankara, Turkey.
** Numune Education and Research Hospital, 2nd Clinic of Neurology, Ankara, Turkey.
° Correspondence

els are consistent findings in patients with Alzheimer's Disease, together with the deterioration of cholinergic neurons [6-20].

Currently, there is a great demand for the development of new drugs to improve memory deficits or to delay the neurodegenerative process. Up to the present, various studies have been focused on constituents with PEP inhibitory activity isolated from plants as well as their synthetic counterparts. In this review, general information is given about PEP and plants possessing PEP inhibitory activity.

Functions of PEP

Proline is unique among 20 amino acids in its cyclic structure. This property imposes many restrictions on the structure of peptides and proteins and confers particular biological properties upon a wide range of physiologically important biomolecules. In order to deal with such peptides, nature has developed a group of enzymes that recognize this residue specifically[21].

Proline-specific peptidases fall into two classes. Prolidase and aminopeptidase P cleave the N-terminal amino acid from a peptide that is followed by proline at the carbonyl-imino bond. Prolinase and proline-imino peptidase cleave proline at the N-terminal position of peptides. Endoproteases that have specificity for proline are HIV-1 protease, prolyl endopeptidase and carboxypeptidase P. HIV-1 protease cleaves the carbonyl-imino bond. PEP and carboxypeptidase P cleave the carboxyl side of the proline residue[22]. As a cytoplasmic protease, PEP has been characterized from several organisms (mushroom, plant, bacteria) and in some organs of various mammals (cow, human, rabbit, pig and rat)[23]. PEP is composed of 705 amino acids including a signal peptide of 20 residues. After cleavage of the signal peptide, the mature protein which is composed of 685 residues is secreted. It is a serine protease of a novel type and does not present a proenzyme form. Although the three dimensional structure of PEP is unknown, a structural pattern composed of several domains has been suggested[24-30]. PEP has also been proposed to be essential for the morphogenetic process of imaginal discs and to participate in DNA synthesis in insect proliferation[31,32].

Inhibitors of PEP

Since PEP can hydrolyze a number of peptide hormones and neurotransmitters, abnormal increases or decreases in PEP activity could result in diseases related to memory and cognition[33-41]. Thus, PEP inhibitors are expected to exert their beneficial effects by increasing the brain levels of those neuropeptides which may improve and restore cognitive functions and protect vulnerable nerves against damage and cell death.

PEP inhibitors may have unique clinical relevance in the prevention and treatment of a wide range of age-related disorders including:

-Memory deficits due to cerebro-vascular sclerosis
-Dementia of Alzheimer's type
-Dementia and cognitive damage secondary to stroke, AIDS etc.

Specific PEP inhibitors are also expected to have anti-amnesic effect. To date, many candidate compounds with PEP inhibitory activity have been synthesized for treatment of the neuropathological disorders mentioned above[42-47].

These studies suggest that PEP inhibitors may improve memory by blocking the metabolism of endogenous neuropeptides and have possible potential for preventing memory deterioration.

PEP Inhibitors Isolated from Plants

Recently, research has been concentrated on plant constituents in order to discover novel PEP inhibitory compounds. For this purpose, sake (alcohol beverage in Japan, made from rice) and its by-product were studied. Pepsin hydrolysates of sake cake as a by-product and sake concentrate were subjected to some chromatographic procedures. Three inhibitory peptides were obtained from sake cake as well as three

other peptides from sake. These peptides which exist in rice glutelin inhibited PEP *in vitro*[48].

In a systematic screening for PEP inhibitors from traditional Chinese medicines, Fan *et al.*[49] found that the methanol extract from the underground portion of *Rhodolia sacra* showed significant inhibitory activity against PEP isolated from *Flavobacterium meningosepticum*. Phytochemical investigation of the extract resulted in the isolation of nineteen known compounds. Six of them, namely protocatechuic acid, gallic acid, (-)-epigallocatechin 3-O-gallate, 3-O-galloylepigallocatechin-(4β → epigallocatechin 3-O-gallate, sacranoside A, arbutin and 4-O-(β-D-glucopyranosyl)-gallic acid showed inhibitory activity. The kinetic study of these inhibitors indicated that they were noncompetitive inhibitors except for protocatechic acid which is a competitive inhibitor.

The same researchers also screened forty-six water and methanol extracts from plants selected on the basis of traditional Chinese medicine for PEP inhibition. Among them, water extracts of *Apocynum venetum* (80.4 %), *Areca catechu* (72.8 %), *Cornus officinalis* (85.3 %), *Polygonum multiflorum* (86.1 %), *Salvia deserta* (58.2 %) and *Uncaria rhynchophylla* (77.4 %) showed more than 50 % inhibition at a concentration of 100 µg/ml, while the methanol extracts of *Anemarrhena asphodeloides* (49.9 %), *Angelica acutiloba* var. *sugiyamae* (60.2 %), *Biota orientalis* (84.3 %), *Eupolyphaga sinensis* (63.3 %), *Ginkgo biloba* (60.2 %), *Lycopodium clavatum* (79.8 %), *Paeonia lactiflora* var. *trichocarpa* (81.7 %), *Paeonia veitchii* (76.9 %), *Polygala tenuifolia* (65.2 %), *Tabanus yao* (57.2 %) and *Ziziphus jojoba* (65.4 %) showed 50 % (≥) inhibition at 100 µg/ml. In addition, the PEP inhibitory activity of the constituents of *Salvia deserta* was investigated together with two positive controls, z-pro-prolinol and z-pro-prolinal. Out of eleven compounds isolated, ten showed inhibitory activity in a concentration-dependent manner[51]. Related to this work, in our study on acetylcholinesterase inhibitory activity of some Turkish plants, we have found that *Lycopodium clavatum* also had inhibitory activity against acetylcholinesterase, which is another enzyme that plays a role in the pathology of Alzheimer's disease[52].

Fan *et al.* also studied the methanol extract of the underground portion of *Rhodolia sachalinensis* for PEP inhibitory activity. From this extract, five new monoterpenoids along with twenty-two known compounds were isolated. Among them, 1,2,3,6-tetra-O-galloyl-β-D-glucose, 1,2,3,4,6,-penta-O-galloyl-_-D-glucose, rhodionin, rhodiosin, 3-O-galloyl epigallocatechin-(4β → -epigallocatechin 3-O-gallate and rosiridin displayed noncompetitive inhibition against PEP[53].

In connection with their work, Fan *et al.*[54,55] also screened PEP inhibitors from fourteen traditional Kampo formulas and found that Tokaku-joki-to (*Persia* and *Rhubarb* combination) showed a significant inhibitory activity. Examination of this formula resulted in the isolation of two new compounds, cis-3,5,4'-trihydroxystilbene 4'-O-β-D-(6-O-galloyl) glucopyranoside (1) and 4-(4-hydroxyphenyl) -2-butanone 4'-O-β-D-(6-O-galloyl-2-O-cinnamoyl) glucopyranoside (2), along with twenty-five known compounds. Twelve of them, namely compounds (1) and (2), 4-(4-hydroxyphenyl)2-butanone 4'-O-β-D-(2,6-di-O-galloyl)glucopyranoside, 4-(4-hydroxyphenyl)-2-butanone 4'-O-β-D-(2-O-galloyl-6-O-cinnamoyl) glucopyranoside, 1,2,6-tri-O-galloylglucose, gallic acid 4-O-β-D-(6-O-galloyl) glucopyranoside, licuroside, (-)-epicatechin, (-)-epicatechin 3-O-gallate caused noncompetitive inhibition.

PEP inhibitory activities of the methanol extracts of a number of Bangladeshi medicinal plants were evaluated by Khanom *et al.* *Embelia officinalis* was found to have a strong inhibitory activity[56]. In addition, *Embelia ribes*, which has been used in Indian folk medicine for various purposes, was found to be active against PEP and embelin was isolated as the compound responsible for the activity[57].

Green tea was also found to have three PEP inhibitors. (-)-Epigallocatechin gallate, (-)-epicatechin gallate and (+)-gallocatechin gallate were identified as the active components in the methanolic extract of green tea leaves[58].

In Anis *et al.*'s[59] work on PEP inhibitory constituents from *Duranta repens*, they isolated five inhibitory compounds, 2 of which were isoprenylated flavonoids, 5,7-dihydroxy-3'-(2-hydroxy-3-methyl-3-butenyl)-3,6,4'-trimethoxyflavone, 3,7-dihydroxy-3'-(2-hydroxy-3-methyl-3-butenyl) - 5,6,4 '-trimethoxyflavone, an isoprenylated acetophenone derivative, 5-hydroxy-3,6,7,4'-tetramethoxyflavone and rosenonolactone.

Kobayashi *et al.*[60] worked on the PEP inhibitory constituents of the roots of *Lindera strychniflora* against PEP from *Flavobacterium meningosepticum* and that from rat brain supernatant. The isolated compounds were identified as the tannins, epicatechin (1) and aesculitannin B (2), as well as the terpene derivatives, namely linderene (3), linderene acetate (4), linderelactone (5), and isolinderalactone (6). Out of these compounds, (1), (2), and (4) inhibited PEP of *F. meningosepticum* origin more strongly than that from rat brain supernatant. However, (3), (5) and (6) inhibited the enzymes from both origins to the same extent. The kinetic study indicated that (1) and (2) were noncompetitive inhibitors while (3)-(6) acted competitively.

Polyozellus multiplex, a mushroom species, exhibited a high PEP inhibitory activity. The ethyl acetate-soluble fraction of *P. multiplex* yielded two active compounds, thelephoric acid and kynapcin-960. The same mushroom species also yielded a series of new benzofuran dimer type compounds (kynapcin-12, -13, -24 and -28) which were shown to inhibit PEP noncompetitively[62-64]. Polyozellin was also identified as another PEP inhibitor compound from *P. multiplex*[65].

Lee *et al.* isolated three noncompetitive PEP inhibitors from *Eugenia caryophyllata* and these compounds were elucidated as luteolin, quercetin and β-sitosterol-3-O-β-D-glucopyranoside. In the same work, twenty authentic flavonoids were tested in order to investigate structure-activity relationship. Only relationships established were the catechol moiety in the B-ring and the 7-OH group in the flavonoid skeleton[66]. In another study by Amor *et al.*,[67] *Syzygium samarangense* afforded nine PEP inhibitory compounds, five of which were of flavonoid-type and four of which were identified as triterpene-type compounds.

CONCLUSIONS

Prolyl endopeptidase (PEP) is a proteolytic enzyme with neuropeptide catabolising activity in the central nervous system. PEP has been reported to be abundant in the hippocampal region of the brain in amnesic patients[1,2]. Therefore, PEP inhibitors are expected to be the remedy for memory dysfunction. The high activity of PEP in the human cortex suggests that PEP could play a role in the functions of this brain area as well.

Since medicinal plants have been proved to be rich sources of biologically active compounds which could be used against the diseases that threaten human health, many recent studies have been carried out on plants in order to find new PEP inhibitors to be primarily used in the treatment of Alzheimer's disease. Any treatment that could positively modulate central neuropeptide levels would provide a promising therapeutic approach to the treatment of cognitive deficits associated with aging and/or neurodegenerative diseases. In conclusion, plants appear to be promising sources of new PEP inhibitors.

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