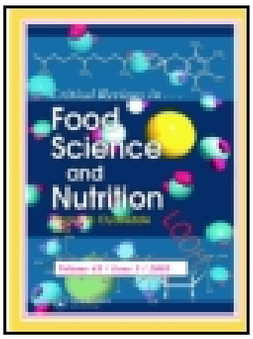

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REVIEW

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The biological role of prolyl oligopeptidase and the procognitive potential of its peptidic inhibitors from food proteins

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ABSTRACT

Prolyl oligopeptidase (POP) is a conserved serine protease belonging to proline-specific peptidases. It has both enzymatic and non-enzymatic activity and is involved in numerous biological processes in the human body, playing a role in e.g., cellular growth and differentiation, inflammation, as well as the development of some neurodegenerative and neuropsychiatric disorders. This article describes the physiological and pathological aspects of POP activity and the state-of-art of its peptidic inhibitors originating from food proteins, with a particular focus on their potential as cognition-enhancing agents. Although some milk, meat, fish, and plant protein-derived peptides have the potential to be applied as natural, procognitive nutraceuticals, their effectiveness requires further evaluation, especially in clinical trials. We demonstrated that the important features of the most promising POP-inhibiting peptides are very short sequence, high content of hydrophobic amino acids, and usually the presence of proline residue.

KEYWORDS

bioactive peptides; food proteins; procognitive peptides; prolyl oligopeptidase; prolyl oligopeptidase inhibitors; structure-activity relationship

Introduction

The progress in medicine has significantly improved life span and quality, but at the same time, it has resulted in the aging of the global society. It is estimated that by 2050 the number of people older than 60 will surpass the population of younger generations, whereas the number of people over 80 will triple – reaching 380 million (Harper 2014). Consequently, the number of individuals suffering from age-related cognitive disorders increases. While over 46.8 million people were affected by dementia in 2015, this number is projected to increase to 131.5 million by 2050 (Prince et al. 2015). No therapy is known to cure the disease. The only clinically available medications for Alzheimer's disease (AD), the most common cause of dementia, are three acetylcholinesterase (EC 3.1.1.7) inhibitors, namely donepezil, rivastigmine, and galantamine, and the N-methyl-D-aspartic acid (NMDA) receptor antagonist memantine. These drugs improve neurotransmission, but they bring only a slight alleviation from the symptoms of AD, and only in some patients (Benek, Korabecny, and Soukup 2020). Therefore, the discovery of appropriate strategies of the cognitive disorders' prevention and treatment is becoming of crucial importance.

Diet has a direct impact on one's cognitive performance (McEvoy et al. 2019; Klimova, Dziuba, and Cierniak-Emerych 2020). Vitamin deficiencies, primarily A, E, C, B, and folates, high consumption of saccharides, and saturated fatty acids are associated with intellectual decline and increased susceptibility to neurological disorders. On the other hand, a diet rich in polyunsaturated fatty acids, especially of n-3 family, polyphenols, and other antioxidants improves cognition and lowers the risk on mental impairments (Beilharz, Maniam, and Morris 2015; Phillips 2017). A change in nutritional habits is often considered to be easier and safer than pharmacotherapy.

Dietary proteins have traditionally been viewed as a source of amino acids. However, reports from a few recent decades indicate that a complementary criterion, allowing a more complete view of the proteins' biological value should also take into account their usefulness as a source of bioactive peptides (Barati et al. 2020). These protein fragments, usually 2-30 amino acid residues in length, are released from protein precursors e.g. during food digestion in the gastrointestinal (GI) tract, fermentation by proteolytic microorganisms, and enzymatic or chemical hydrolyzis. They can also be obtained by chemical synthesis or through the expression of corresponding genes (Zambrowicz et al. 2013; Iwaniak, Darewicz, and Minkiewicz 2018). Biopeptides not only serve as nutrients but can also exert drug-like properties. The most extensively studied food-derived bioactive peptides are angiotensin-converting enzyme (ACE; EC 3.4.15.1) inhibitory, dipeptidyl peptidase IV (DPP IV; EC 3.4.14.5) inhibitory, and antioxidative peptides. Some of the biopeptides, which efficiency and safety were confirmed in human studies, are applied as active ingredients in functional foods. Examples of commercial products containing bioactive peptides include: Calpis® (Calpis Co. Ltd., Japan), Evolus® (Valio Ltd., Finland), BioZate® (Davisco Foods, USA), and

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Lapis Support (Tokiwa Yakuhin Co. Ltd., Japan) (Iwaniak, Darewicz, and Minkiewicz 2018).

Conventionally, the effects of dietary proteins and peptides on the functions of the central nervous system (CNS) have mainly been attributed to their role as a source of amino acids, acting as precursors of important neurotransmitters, including serotonin, dopamine, epinephrine, and norepinephrine (Van De Rest, Van Der Zwaluw, and De Groot 2013). In recent years though, an increasing number of the whole peptide sequences with cognition-affecting activity have been discovered. Examples include opioid peptides that exert diverse regulatory functions associated with emotion and memory (Z. Liu and Udenigwe 2019), and various neuroprotective peptides, such as acetylcholinesterase inhibitory, antioxidative, anti-inflammatory and immunomodulatory (Lemieszewska et al. 2016; S. Y. Lee and Hur 2019). The cognition-enhancing effects can also be exhibited by peptides showing prolyl oligopeptidase (POP) inhibitory properties, last reviewed over 11 years ago (Wilson, Hayes, and Carney 2011). The purpose of this study is to describe the physiological and pathological aspects of POP activity in the human body and the potential of its peptidic inhibitors derived from food proteins with a focus on their procognitive properties.

Prolyl oligopeptidase and its role in human pathophysiology

Classification, occurrence and structure

Prolyl oligopeptidase (EC 3.4.21.26) also known as prolyl endopeptidase, proline endopeptidase, proline specific endopeptidase, post-proline endopeptidase, endoprolylpeptidase, and post-proline cleaving enzyme is a conserved serine protease belonging to proline-specific peptidases (PSP). PSPs are the only proteolytic enzymes capable of catalyzing the hydrolyzis of peptide bonds formed by Pro residues, except for a few nonspecific metallopeptidases that can hydrolyze such bonds, providing that they are present at peptides' N-terminus (Dunaevsky et al. 2020). Most of the known POPs have demonstrated the ability to hydrolyze only short peptides, up to approx. 30 amino acid residues in length, hence the name prolyl oligopeptidase (Svarcbahs et al. 2019).

POP was first detected in the human uterus as a protease responsible for oxytocin degradation via cleavage of Pro-Leu peptide bond (Walter et al. 1971). It is a soluble, cytoplasmic enzyme, but under inflammatory conditions it can also be secreted into the extracellular matrix (Natunen et al. 2019). The human POP is distributed broadly in numerous body parts including kidneys, thymus, testis, muscles, heart, and the CNS (Dunaevsky et al. 2020). In the brain, the majority of POP expression and activity has been detected in neurons of the brain cortex and nigrostriatal system, particularly in substantia nigra, caudate nucleus, and pallidum (Irazusta et al. 2002; Myöhänen et al. 2007). The immunoreactive POP protein, POP mRNA, and POP activity levels are not always correlated, most likely due to the presence of endogenous POP inhibitors, and other PSPs in the examined tissues, exhibiting similar specificity while being resistant to POP specific inhibitors (Bracke et al. 2019; Svarcbahs et al. 2019). The difficulty in confirming the data on POP distribution is additionally influenced by the unknowingness of its specific antibodies (Svarcbahs et al. 2019) and the fact that studies on this protease were performed using various methods, under different conditions (Dunaevsky et al. 2020).

The human POP is composed of 710 amino acid residues, with a molecular weight of approximately 80 kDa, almost three times higher than classical serine proteases (trypsin and chymotrypsin). This enzyme is a cylindrically shaped, monomeric protein, consisting of two domains. The first is catalytic, with an α/β hydrolase fold, and the second, unique β-propeller, is composed of a seven-fold repeat of four-stranded β sheets. The active center resides at the interface of the domains, in the cavity gorge. The order of amino acid residues forming the catalytic triad is unusual. In classical serine proteases it is Asp-His-Ser or His-Asp-Ser, while in POP it is Ser-Asp-His (Dunaevsky et al. 2020). Another unique feature of POP is a system of flexible loops that is vital for its enzymatic activity. The system is comprised of a loop of the noncatalytic domain (called loop A), a loop of the catalytic domain (called loop B), and other catalytically important loops. The system controls the proteolysis via motion and rearrangement of the loop structure. It regulates ligand entry and binding to the active site, thus influencing the enzyme's specificity, and is stabilized by inhibitor binding. It has also been suggested to be a promising target for inhibitor design (Szeltner et al. 2013; Tsirigotaki et al. 2017). The 3 D structure of human POP, accessed from the UniProt database (The UniProt Consortium 2019), is presented in Figure 1.

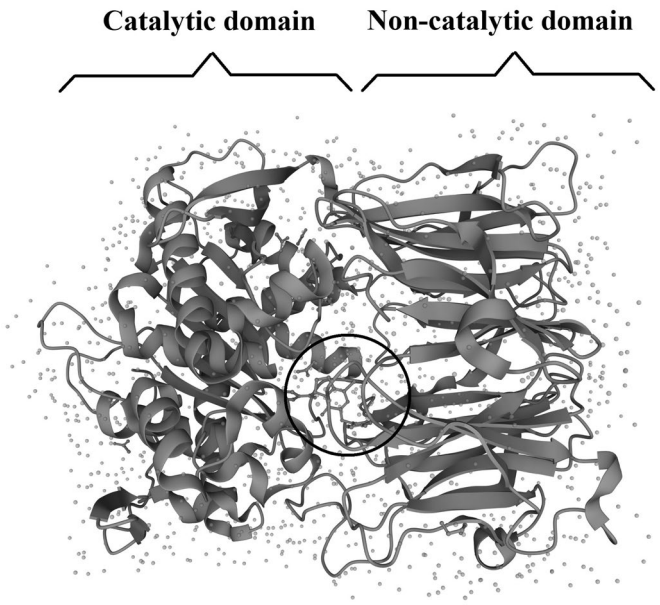

Figure 1. The 3 D structure of human POP with the inhibitor (6 s)-1-chloro-3-[(4-fluorobenzyl)oxy]-6-(pyrrolidin-1-ylcarbonyl)pyrrolo[1,2-a]pyrazin-4(6h)-one bound at the active stie (circled) [the UniProt database (accession date: 22.12.2022)].

Enzymatic activity

Most PSPs are classified as exopeptidases, with POP and fibroblast activation protein being the only exceptions, exhibiting endopeptidase activity (Dunaevsky et al. 2020). POP breaks the peptide bonds at the carboxyl side of Pro, with a recognition sequence of X-Pro-Y, where X is a peptide or protected amino acid, and Y is a peptide, an amino acid, an amide, an alcohol, or an aromatic amine (Walter, Simmons, and Yoshimoto 1980). Early studies on the specificity of this enzyme have suggested that the noncatalytic domain acts as a gate to the active site that limits the access of substrates larger than approx. 30 amino acids. However, a few POPs capable of hydrolyzing longer peptides, as well as intact proteins, have been reported (Kang, Yu, and Xu 2014). Therefore, the major factor influencing the POP specificity is substrate's accessibility to the enzyme's active site, rather than its chain length.

POP is responsible for the maturation and degradation of short, Pro-containing neuropeptides and hormones, including substance P, thymosin β4, thyrotropin-releasing hormone, and arginine-vasopressin that are known to be important modulators of cognitive processes. POP activity is also associated with several neurological and mental disorders. This is why, despite the ubiquitous localization of POP in the body, the enzyme found in the CNS is the most important therapeutic target (García-Horsman, Männistö, and Venäläinen 2007). POP also participates in angiotensin processing, therefore it is a member of the renin-angiotensin-aldosterone system – one of the major physiological mechanisms for the maintenance of water-electrolyte homeostasis and regulation of blood pressure (Serfozo et al. 2020). The hydrolytic activity of POP is measured using various chromogenic and fluorogenic substrates such as Z-Gly-Pro-Leu-Ala (Yoshimoto et al. 1988), Z-Gly-Pro-Leu-Gly-Pro (Sattar et al. 1990), or Z-Gly-Pro-4-nitroanilide (Kang, Yu, and Xu 2014).

Non-enzymatic activity

Although customarily the main biological function of POP had been considered to be related to its hydrolytic properties, investigations have revealed that peptide hydrolyzis alone cannot fully explain the enzyme's role in the processes in which it was relevant. Therefore, recent studies have paid more attention to the enzyme's non-catalytic activity resulting from POP's involvement in direct, physiologically important protein-protein interactions (PPIs) (Männistö and García-Horsman 2017). The most important of POP's binding partners include α-tubulin, neurospecific growth-associated protein-43 (GAP-43), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and α-synuclein (αS) (Dunaevsky et al. 2020). The strength of the PPIs depends on the protease's conformation, which in turn is influenced by the presence of its ligand. It was also speculated that POP enzyme-substrate complex might require the PPIs to form an enzymatically active tertiary complex (Männistö and García-Horsman 2017). However, the protease's conformational dynamics and its relevance in the enzyme's role are not yet fully elucidated, as even catalytically inactive POP mutants were reported to maintain some of its' regulatory functions (Svarcbahs et al. 2019). More details on the significance of these PPIs with particular proteins is described in the following sections.

Activity in cellular growth

POP is engaged in age-dependent processes. Studies on rodents demonstrated that POP mRNA levels are the highest during perinatal growth and at birth, then progressively decline as the animals age (Agirregoitia et al. 2010), to greatly increase again in old age (C. H. Jiang et al. 2001). The rise of POP levels in early life stages is likely due to its involvement in proliferation and differentiation of various types of cells, including liver (Matsubara et al. 1998), ovary (Kimura et al. 1998), testis (Kimura, Matsui, and Takahashi 2002) and neurons (Moreno-Baylach et al. 2008; Hannula, Männistö, and Myöhänen 2011). According to Höfling et al. (2016), the mice with the POP gene knocked out manifested alterations in brain development including reduced volume of the brain cortex and disruption in synaptic plasticity. They also demonstrated increased expression of some neuropeptides receptors and polysialylated-neural cell adhesion molecule, an important mediator of neuroplasticity, while exhibiting reduced anxiety but increased activity similar to attention deficit hyperactivity disorder (ADHD). D'Agostino et al. (2013) reported that the POP- gene-trapped mice exhibited a decrease in hippocampal synaptic spine density and long-term potentiation, impairment in hippocampal-mediated learning, and reduced levels of GAP-43 that is involved in axon guidance and growth cone formation. Di Daniel et al. (2009) described the ability of POP to form PPI with GAP-43, independently of the enzyme's catalytic activity, and the possibility of fixing the alternations in growth cone dynamics of POP knock-out cells via transfection of either wild type or catalytically inactive POP mutant.

Role in inflammation

POP is linked to inflammatory processes. Along with other proteases capable of hydrolyzis of bigger proteins it generates immunoactive peptides: N-acetyl-Pro-Gly-Pro from endogenous collagen and N-acetyl-Ser-Asp-Lys-Pro from thymosin β4, both exerting anti-inflammatory properties and acting as markers of inflammation. The level of the former peptide was correlated with POP levels in murine model of inflammatory pulmonary diseases. Additionally, POP levels were increased in the brain of mice after inflammatory insult (Penttinen et al. 2011). The POP-deficient mice have shown no inflammatory response to lipopolysaccharide treatment, unlike wild-type animals (Höfling et al. 2016), and the POP gene-disrupted mice exhibited decreased intensity of inflammatory responses to a high-fat diet (D.-X. Jiang et al. 2020). Furthermore, Roda et al. (2014) suggested that the model of valproic acid (VPA) interaction with POP indicates a promising possibility of using POP inhibitors as therapeutics in inflammatory disorders.

Significance in neuropsychiatric disorders

The POP plasma activity level is altered in people affected by neuropsychiatric disorders. It is reduced in individuals suffering from anorexia, bulimia nervosa (Maes et al. 2001), and depression, but increased in those with mania, schizophrenia (Maes et al. 1995), and post-traumatic stress disorder (Maes et al. 1999). Significant alterations of POP plasma activity, that is higher mean and standard deviation, were observed in children with autistic spectrum disorders as compared to the non-autistic group (Momeni et al. 2005).

García-Horsman, Männistö, and Venäläinen (2007) described two theories relating the POP activity to neuropsychiatric disorders. The first is connected to several behavior-modulating neuropeptides that are involved in the pathophysiology of depression, i.e. thyrotropin-releasing hormone and arginine-vasopressin, which levels are modified by altered POP activity. The second is related to POP's association with the metabolism of inositol 1,4,5-triphosphate (IP3), a vital cellular second messenger in the cascade of neuropeptide signaling. Schulz et al. (2002) reported that the level of this protease's expression was inversely correlated to IP3 concentration and POP inhibition enhanced substance P-mediated stimulation of IP3 production. Interactions of POP with other enzymes controlling IP3 level were also described by other authors (Williams et al. 1999; Harwood 2011).

POP inhibition was shown to reverse the effect of three traditional mood-stabilizing drugs: VPA, lithium, and carbamazepine, all exerting IP3 depleting activity (Williams et al. 2002). Concurrently, VPA was demonstrated to be a POP inhibitor itself. It might explain VPA's ability to treat both mania and depression, assuming that steady mood is affected by stable IP3 signaling – which can be both reduced by VPA treatment due to its IP3 diminishing activity and elevated via the drug's POP inhibiting properties (Cheng et al. 2005). This theory is consistent with the previous study, which revealed that antidepressants and VPA restore plasma POP activity to near control levels in depressed and manic patients, respectively (Maes et al. 1995).

Relevance in neurodegeneration

POP is involved in several neurodegenerative disorders, including dementia with Lewy bodies, AD, Parkinson's disease, Huntington disease, and multiple sclerosis (García-Horsman, Männistö, and Venäläinen 2007; Svarcbahs et al. 2019; Trallero et al. 2019). Although the precise etiology of these pathologies is unknown, common to them is chronic oxidative stress (Singh et al. 2019) and accumulation of insoluble aggregates of misfolded proteins in neurons, ultimately resulting in their death (Gandhi et al. 2019).

In *post mortem* brains of patients with AD and Parkinson's disease, POP strongly colocalized with three proteins susceptible to the pathological misfolding and directly involved in the neurodegenerative diseases, i.e. αS, β-amyloid (βA), and τ-protein (Hannula et al. 2013). These proteins often co-occur, accelerate each other's aggregation and share several protein interactors (Irwin and Hurtig 2018; Yan, Uronen, and Huttunen 2020).

POP was suggested to be one of γ-secretases, i.e. enzymes responsible for the generation of neurotoxic peptides from βA, as POP inhibition reduced the generation of βA in neuroblastoma cells and hindered βA-like deposition in the brain of mice used as a model of accelerated senescence (Barelli et al. 1999).

Moreover, POP lack or inhibition resulted in a decrease in reactive oxygen species (ROS) production. Puttonen et al. (2006) demonstrated that POP inhibitors are able to prevent some neurotoxin-induced cell stress-related factors, such as ROS production and nuclear translocation of GAPDH which was suggested to be a proapoptotic mediator and intracellular sensor of oxidative stress. According to Dokleja, Hannula, and Myöhänen (2014), POP inhibition improve the viability of SH-SY5Y human neuroblastoma cells by decreasing ROS production via the reduction of αS aggregate formation. Svarcbahs et al. (2018) used POP knock-out cells and mice to show that POP enhances αS-mediated toxicity both in vitro and in vivo, as well as impairs the ability of proteasomal systems to degrade it.

Role in autophagy and cancer

POP intensifies aggregation of αS via PPI and negatively regulates its autophagy, one of the major catabolic mechanisms of dysfunctional component removal. These processes are dampened by POP inhibitors, although this dampening effect's intensity for particular inhibitors is not correlated with their inhibitory potency, i.e. IC_{50} values (half-maximal POP inhibitory concentrations) (Kilpeläinen et al. 2020). POP regulates autophagy via negative regulation of protein phosphatase 2 A, a regulator of cell cycle and growth, which reduced levels lead to aggregation of αS and τ-protein hyperphosphorylation resulting in AD, and disturbances in cell proliferation, inducing carcinogenesis (Svarcbahs et al. 2020).

POP protein and activity levels are elevated in tumors. In several cancer cell lines, i.e. neuroblastoma, gastric cancer, and human breast cancer, in which protein phosphatase 2 A activity alterations are seen, POP inhibition resulted in a reduction of the cell proliferation rate. The mechanism of anticancer activity is not precisely examined, although it is known that G_0/G_1 arrest is involved (Sakaguchi et al. 2011; Suzuki et al. 2014; S. Tanaka, Kanayo, and Sakaguchi 2017).

POP inhibitors from food proteins

Several synthetic POP inhibitors have been developed, potent and selective, with IC_{50} values reaching the nanomolar range. Some of them have been experimentally proven to be efficacious cognition-enhancing agents, even in clinical trials. However, none has entered the pharmaceutical market so far, due to insufficient information on their pharmacokinetics, pharmacodynamics, bioavailability, toxicity, and incomplete understanding of the POP's physiological role and its association with pathological conditions (Babkova et al. 2017; García-Horsman 2020). The majority of strong POP

inhibitors are low molecular weight, substrate-like peptidic molecules based on N-acyl-L-prolyl-pyrrolidine scaffold. Usually, the P1 site is a pyrrolidine ring with an electrophile in its 2S-position, the P2 site is an α-aminoacyl group, and the P3 site is a hydrophobic acyl group (Kilpeläinen et al. 2020). The first discovered POP inhibitor was N-benzyloxycarbonyl-L-prolyl-L-prolinal (i.e. Z-pro-prolinal) (Wilk and Orlowski 1983). Some peptides derived from food proteins also exhibit POP inhibitory activity (Wilson, Hayes, and Carney 2011). Sequences of 63 such peptides are deposited in the BIOPEP-UWM database (accession date: 11.01.2023), each containing at least one Pro residue. The full list of these peptides is available in the database when searching for compounds with "antiamnestic" activity (Minkiewicz, Iwaniak, and Darewicz 2019).

Milk protein-derived POP inhibitors

Among the food proteins, the proteins of milk have been the most widely studied as precursors of bioactive peptides. POP-inhibiting peptides have been detected in milk or milk products obtained from various mammalian species, including cows, sheep, and humans. Examples of milk protein-derived peptides with POP inhibitory properties, and other bioactivities of peptides with the same sequence (accessed from the BIOPEP-UWM database on 10.01.2023) are shown in Table 1.

In silico studies

A theoretical study based on BIOPEP-UWM revealed that bromelain and papain may release peptides containing POP inhibiting motifs from bovine α_{S1}- and β-casein (Iwaniak and Taraszkiewicz 2022).

In vitro studies

The first evidence of milk protein-derived POP inhibitors was provided by Asano, Nio, and Ariyoshi (1991) and Asano, Nio, and Ariyoshi (1992) who synthesized various Pro-containing peptides and peptide derivatives sharing sequence homology with human, bovine, buffalo, and ovine β-caseins. Their POP repressive properties were sequence-dependent. The majority of the most effective peptides contained hydrophobic amino acid residues, giving them easy access to the enzyme's active site. Pripp (2006) used the data about the sequences and the IC_{50} values of these peptides to create a quantitative structure-activity relationship model. It demonstrated that hydrophobicity and molecular bulkiness of amino acids in positions P3, P2, and P1' of inhibitory sites of the peptides were positively correlated with their POP inhibitory potency. Several of these peptides were released during simulated GI digestion of both raw and pasteurized human milk (Wada and Lönnerdal 2015).

Sørensen et al. (2004) found potent POP inhibitory peptides in water-soluble extracts from Cheddar, Norvegia, Jarlsberg, and Blue cheeses. These peptides were characterized by molecular weight in the range of 1 to 3.5 kDa and diverse hydrophobicity. Similarly, Srinivas and Prakash (2010) reported that bovine α-casein hydrolyzate by chymotrypsin expressed POP inhibitory activity with IC_{50} at 1.3 mg/mL. According to Öztürk and Akın (2021), several POP inhibiting peptides were released during 180 days of Tulum cheese ripening.

Hsieh et al. (2016) used thermolysin, bromelain, and the GI enzymes to hydrolyze sodium caseinate. All of the obtained hydrolyzates exhibited POP repressing properties. The most potent activity was shown by hydrolyzate prepared by bromelain. Nine oligopeptidic α-, β-, and κ-casein-derived competitive POP inhibitors were identified (see Table 1).

It used to be believed that Pro residue is necessary for POP inhibition, therefore the majority of studies aiming to discover them were focused on Pro-rich proteins or peptides. Sistla (2013) demonstrated that Pro-free peptides Gln-Lys-Ala-Leu-Asn-Glu-Ile-Asn-Gln-Phe and Thr-Lys-Lys-Thr-Lys-Leu-Thr-Glu-Glu-Glu-Lys-Asn-Arg-Leu from α_s-casein also exhibit POP inhibitory activity (see Table 1).

The first study to reveal the ability of casein-derived peptides to inhibit POP from human cells was reported by Juillerat-Jeanneret, Robert, and Juillerat (2011). The authors established that bovine milk caseins fermented by lactic acid

Table 1. Milk protein-derived peptides exhibiting POP inhibitory activity in vitro.

Source	Peptide sequence	IC_{50} [μM]	Other bioactivities	References
human β-casein	Ile-Tyr-Pro-Phe-Val-Glu-Pro-Ile	8	n. a.	(Asano, Nio, and Ariyoshi 1991)
bovine α_{S1}-casein	Phe-Val-Ala-Pro-Phe-Pro-Glu-Val-Phe-Gly	60	n. a.	(Juillerat-Jeanneret, Robert, and Juillerat 2011)
bovine α_{S2}-casein	Gln-Lys-Ala-Leu-Asn-Glu-Ile-Asn-Gln-Phe	0.0058	n. a.	(Sistla 2013)
bovine α_{S2}-casein	Thr-Lys-Lys-Thr-Lys-Leu-Thr-Glu-Glu-Glu-Lys-Asn-Arg-Leu	0.036	n. a.	(Sistla 2013)
bovine β-casein	Asn-Leu-His-Leu-Pro-Leu-Pro-Leu-Leu	150	anticancer	(Juillerat-Jeanneret, Robert, and Juillerat 2011)
bovine β-casein	Leu-Pro-Pro	875	ACE inhibitor	(Siltari et al. 2012; Martin and Deussen 2019)
bovine β-casein	Val-Pro-Pro	761	ACE inhibitor, anti-inflammatory	(Siltari et al. 2012; Chakrabarti and Wu 2015; Martin and Deussen 2019)
bovine κ-casein	Ile-Pro-Pro	486	ACE inhibitor, anti-inflammatory, α-amylase inhibitor, α-glucosidase inhibitor	(Siltari et al. 2012; Chakrabarti and Wu 2015; Martin and Deussen 2019; Martini et al. 2021)
bovine sodium caseinate	Pro-Ile-His-Asn-Ser-Leu-Pro-Gln-Asn-Ile-Pro-Pro-Leu-Thr-Gln-Thr-Pro-Val	29.8	n. a.	(Hsieh et al. 2016)
bovine lactoferrin	Ser-Val-Asp-Gly-Lys-Glu-Asp-Leu-Ile-Trp	1808.4	n. a.	(Manzanares et al. 2018)
bovine lactoferrin	Ac-Arg-Lys-Trp-His-Phe-Leu-Trp-NH_2	296.4	n. a.	(Manzanares et al. 2018)

n. a. – no activity according to the BIOPEP-UWM database.

bacteria contain peptides capable of inhibiting several enzymes present in extracts of HT-29 and SW480 human colon carcinoma cells, including POP. However, the peptides exhibited no such activity in the intact cells, as they were probably not absorbed. These peptides were characterized by a lack of cytotoxicity and IC_{50} values in the range of 30 to 250 µM. The concentration of these peptides in fermented milk can exceed these values (Juillerat-Jeanneret, Robert, and Juillerat 2011).

In vivo studies

Although not confirmed to have POP-inhibiting activity, Colostrinin™ – a complex of low molecular weight (0.5-3 kDa), Pro-rich (about 20%) peptides isolated from ovine, bovine, caprine, and human colostra was shown to have procognitive properties (Sokołowska et al. 2008). According to Stańczykiewicz et al. (2017), consumption of Colostrinin™ at the dose of 4 µg had beneficial effects on the cognitive functions of young rats. Lemieszewska et al. (2018) isolated a peptide with sequence Arg-Pro-Lys-His-Pro-Ile-Lys-His-Gln, sharing sequence homology with sheep and beef αS1-caseins, from Colostrinin™ and demonstrated its anti-apoptotic and neuroprotective activity in adrenal pheochromocytoma PC12 cells. Colostrinin™ was also shown to improve the cognitive performance of patients with moderate AD (Bilikiewicz and Gaus 2004).

Manzanares et al. (2018) were the first to reveal that peptides obtained via hydrolyzis of lactoferrin, glycoprotein from milk whey, and a few sequence-related synthetic peptides, exhibited POP repressive properties in vitro. They also protected transgenic nematode *Caenorhabditis elegans*, a convenient in vivo model used in screening for potential AD drugs, from the toxicity caused by βA-derived peptides. According to the authors, the protective effect was not related to antioxidative activity, as these peptides did not show such properties, instead the importance of tryptophan residue for the bioactivity was highlighted.

The most widely studied ACE inhibitory peptides from milk proteins are so-called lactotripeptides: Leu-Pro-Pro and Val-Pro-Pro from β-, and Ile-Pro-Pro from κ-casein. Their bioavailability and antihypertensive properties were confirmed in numerous animal and human studies, and they are the active ingredients of nutraceutical drinks, e.g. Calpis® (Calpis Co. Ltd., Japan), Evolus® and Valio® (Valio Ltd., Finland) (Iwaniak, Darewicz, and Minkiewicz 2018). These peptides were also identified in cheeses, although their concentration varies significantly between different cheese types (Iwaniak and Mogut 2020). Siltari et al. (2012) studied whether any other vascular function regulating enzymes are also inhibited by the lactotripeptides and found that these molecules are POP inhibitors, unlike the free amino acids of which they are composed. Human studies revealed that the consumption of lactotripeptides promotes cognitive performance. According to Hamasaki et al. (2019), it improved brain neural activation related to enhanced cognitive function and decreased arterial stiffness in middle-aged and older adults, regardless of their involvement in physical activity. Akazawa et al. (2018) reported that it increased middle cerebral blood flow velocity.

Although an inverse correlation between milk consumption and the frequency of cognitive disorders was shown in meta-analyses, which are placed at the top of pyramid in the evidence-based medicine (J. Lee et al. 2018; Wu and Sun 2016), the general evidence is not strong enough to draw a solid conclusion on that subject. This is due to several significant study limitations, including methodological variability and a limited number of high-quality research papers.

Meat and fish protein-derived POP inhibitors

Several POP-inhibiting peptides have been discovered in meat and fish proteins, mostly in collagen and a few in other proteins. The examples of such peptides, along with other bioactivities of peptides with the same sequence (accessed from the BIOPEP-UWM database on 10.01.2023) are shown in Table 2.

In silico studies

Minkiewicz, Dziuba, and Michalska (2011) reported that proteinase K and proteinase P1 can potentially release various biopeptides from bovine muscle proteins, including POP inhibitors. According to these authors, among the bovine proteins analyzed, collagen and elastin were found

Table 2. Meat protein-derived peptides exhibiting POP inhibitory activity in vitro.

Source	Peptide sequence	IC_{50} [µM]	Other bioactivities	References
collagen	Pro-Gly	n. d.	ACE inhibitor, antithrombotic, DPP IV inhibitor, regulating the stomach mucosal membrane activity	(Cheung et al. 1980; Ashmarin et al. 1998; Lan et al. 2015)
collagen	Gly-Pro	n. d.	ACE inhibitor, antithrombotic, DPP IV inhibitor, regulating the stomach mucosal membrane activity	(Yoshimoto et al. 1978; Ashmarin et al. 1998; Byun and Kim 2002)
bovine collagen	Pro-Pro-Gly	2700	DPP IV inhibitor	(Lafarga, O'Connor, and Hayes 2014; Lafarga, O'Connor, and Hayes 2015)
collagen	Pro-Gly-Pro	n. d.	antithrombotic, chemotactic, inhibitor of insulin secretion, regulating the stomach mucosal membrane activity	(Ashmarin et al. 1998; O'Reilly et al. 2009)
bovine serum albumin	Pro-Pro-Leu	2860	ACE inhibitor, DPP IV inhibitor	(Wang et al. 2011; Lafarga, O'Connor, and Hayes 2014; Lafarga, O'Connor, and Hayes 2015)
porcine and bovine myosin	Ala-Pro-Pro-His	3950	ACE inhibitor	(Lafarga, O'Connor, and Hayes 2014; Lafarga, O'Connor, and Hayes 2015)

n. d. – not determined.

to be the best sources of such peptides, due to the high content of Pro and Gly residues. Lafarga, O'Connor, and Hayes (2015) synthesized a few POP inhibitory peptides which they had predicted *in silico* to be released from meat proteins by various proteases. These peptides were characterized by diverse inhibitory activity (see Table 2), resistance to further degradation by the GI enzymes and lack of toxicity, according to *in silico* analyses. B.-B. Huang, Lin, and Chang (2015) identified 7 proteins in extracts of tilapia frame and skin, and established that their theoretical hydrolyzates by various proteases contain POP inhibiting peptides. Kęska and Stadnik (2016) found that several POP inhibitory peptides can potentially be released from porcine myofibrillar proteins hydrolyzed by GI enzymes, i.e. pepsin, trypsin, and chymotrypsin. Collagens from cow, pig, sheep, chicken, duck, horse, salmon, rainbow trout, goat, rabbit, and turkey hydrolyzed *in silico* with various proteases were found out to be theoretically promising sources of POP inhibiting peptides, whose frequency of occurrence in the collagens' sequences was only inferior to ACE and DPP IV inhibitory peptides (Iwaniak et al. 2020). Adopting a similar approach, based on the BIOPEP-UWM database, we predicted that some POP inhibitory peptides can be released during enzymatic hydrolyzis of chicken feather and pig hair keratins (Taraszkiewicz et al. 2022).

In vitro studies

Sørensen et al. (2004) reported that hydrolyzates of cod, salmon, and trout muscle proteins produced by endogenous and/or exogenous proteases contain diverse peptides exhibiting strong POP inhibitory activity. According to Sila et al. (2015), barbel skin gelatin hydrolyzates produced by various proteases contained POP and DPP IV inhibiting peptides, the potency of which was sequence-dependent and length-independent.

Similarly, hydrolyzates of proteins from sardine and tuna head, muscle, and viscera prepared by Alcalase contained <3 kDa peptides with moderate POP and ACE inhibitory properties (Martínez-Alvarez et al. 2016). The bioactivity of these peptides was enhanced considerably after simulated gastric digestion, due to the release of smaller peptides, while remaining unchanged following continued intestinal digestion (Martínez-Alvarez et al. 2016).

Lajmi et al. (2019) studied the effect of hydrolyzis conditions by trypsin on the release of ACE and POP inhibitory peptides from proteins found in smooth hound collagenous by-products, i.e. skin, tail, and fins. The authors reported that the optimal pH and temperature of the process affect the desired bioactivity of peptides to be produced because the most potent POP inhibitory activity was obtained in the hydrolyzate produced at 35 °C at pH 7, whereas the greatest ACE inhibition was exhibited by the hydrolyzate obtained at 45.9 °C at pH 9.

POP inhibitory peptides were also detected in proteins derived from meat offal. Ohmori et al. (1994) isolated such peptide with sequence Met-Pro-Pro-Pro-Leu-Pro-Ala-Arg-Val-Asp-Ala-Leu-Asn from bovine brain. Papain, collagenase, and Alcalase generated POP repressing peptides from heat- and pressure-treated proteins found in bovine lungs, which inhibitory potency was dependent on the enzyme used and method of pretreatment applied (Lafarga and Hayes 2017).

In vivo studies

Porcine brain hydrolyzate exhibited POP inhibiting properties and improved cognitive performance of βA-peptide-infused rats (Y.-T. Liu et al. 2019).

Collagen is a rich source of bioactive peptides, POP inhibitors in particular, owing to a unique structure of this protein, comprising of a repetitive sequential motif of Gly-Pro-Hyp (Iwaniak et al. 2020). A set of so-called glyprolines, i.e. short peptides derived from this motif, including Pro-Gly, Gly-Pro, Pro-Gly-Pro, Gly-Pro-Gly-Gly, and cyclic Pro-Gly, inhibit POP and potentiate memory consolidation processes in the CNS (Ashmarin et al. 1998). Besides, the glyprolines show a variety of other bioactive properties, both positive and negative, depending on their structure and organ or system they affect, including antithrombotic, anti-inflammatory, chemoattractive, hemostasis regulatory, and neuroprotective (Misiura and Miltyk 2019). Human studies revealed that following collagen hydrolyzate ingestion, several short Pro-containing peptides were quickly absorbed into the blood where they remained for a long time, which confirms their bioavailability (Sato, Jimi, and Kusubata 2019; Koizumi et al. 2019).

Collagen hydrolyzates have shown some promising results in improving cognitive capabilities in in vivo studies. They were reported to improve the recovery rate of rats suffering from surgical brain injury by angiogenesis promotion (K.-F. Huang et al. 2014) and exhibit neuroinflammation lowering properties in these animals (Chen et al. 2019). They also enhanced spatial memory and learning, reduced the oxidative stress, and upregulated the expression of brain-derived neurotrophic factor in aged mice (Pei et al. 2010), as well as induced changes in the human brain, along with improvements in language cognitive functions (Koizumi et al. 2019). However, most likely due to a limited number of studies, no systematic reviews have assessed the collagen's effectiveness in preventing mental impairments.

Findings from the latest systematic review regarding meat consumption's effects on cognitive function were inconsistent (Zhuang et al. 2020). Meta-analysis of several studies indicated some protective effect of meat consumption toward cognitive disorder's frequency but no strong conclusions can be currently drawn due to limited representativeness and possible publication bias (Zhuang et al. 2020).

Plant protein-derived POP inhibitors

Plant-based bioactive peptides were investigated less extensively than those from proteins of animal origin. However, some examples of POP inhibiting peptides have been found in plant-derived proteins. Sequences of several POP inhibitory peptides originating from plant proteins are presented in Table 3.

Table 3. Plant protein-derived peptides exhibiting POP inhibitory activity in vitro.

Source	Peptide sequence	IC_{50} [μM]	References
corn γ-zein	His-Leu-Pro-Pro-Pro-Val	80	(Maruyama et al. 1992)
corn γ-zein	Leu-Pro-Pro-Val	240	(Maruyama et al. 1992)
soybean cell wall protein	Lys-Pro-Pro-Ile	380	(Maruyama et al. 1992)
soybean cell wall protein	Lys-Pro-Pro-Val	270	(Maruyama et al. 1992)
red wine	Val-Glu-Ile-Pro-Glu	17	(Yanai, Suzuki, and Sato 2003)
rice glutelin	pGlu-Leu-Phe-Asn-Pro-Ser-Thr-Asn-Pro-Trp-His-Ser-Pro	24.3	(Saito et al. 1997)
rice glutelin	Ser-Pro-Phe-Trp-Asn-Ile-Asn-Ala	42.8	(Saito et al. 1997)
cocoa seed protein	Asn-Tyr-Asp-Asn-Ser-Ala-Gly-Lys-Trp	483.8	(Martorell et al. 2013)
cocoa seed protein	Asp-Asn-Tyr-Asp-Asn-Ser-Ala-Gly-Lys-Trp-Trp-Val-Thr	122.1	(Martorell et al. 2013)

In silico studies

In silico analysis of cereal storage proteins by Cavazos and Gonzalez de Mejia (2013) revealed that some POP inhibitory peptides occur within sequences of wheat gliadin and glutenin, barley hordein, oat globulin, and rice glutelin. According to Amin et al. (2022), POP inhibiting peptides can theoretically be released from large subunit of RuBisCO from green seaweed *Ulva lactuca* by the action of pancreatic elastase, chymotrypsin C, papain, ficin, bromelain, calpain 2, and pepsin.

In vitro studies

Maruyama et al. (1992) synthesized several peptides with sequences homologous to fragments of several Pro-rich proteins, i.e. corn γ-zein, soybean cell wall protein, and carrot 33-kDa protein. They also hydrolyzed the γ-zein using 10% (E/S) subtilisin in an attempt to generate POP inhibitors. The obtained peptides were characterized by diverse, sequence-dependent inhibitory potency (see Table 3).

The POP inhibiting peptides were also discovered in some alcoholic drinks. Saito et al. (1997) isolated some POP inhibitory peptides, originating from rice glutelin, from sake, a traditional Japanese alcoholic beverage, and sake by-product (sake cake) hydrolyzate by pepsin (see Table 3). Yanai, Suzuki, and Sato (2003) obtained two POP inhibitory oligopeptides from proteins present in grape juice and wine (see Table 3). These peptides were also able to reduce the degradation rate of memory-related neuropeptides: arginine-vasopressin, substance P, and neurotensin in vitro. The authors found that the concentration of these peptides in the analyzed materials depends on the grape kind and the winemaking conditions.

Hsieh et al. (2016) used thermolysin, bromelain, and the GI enzymes to obtain hydrolyzates of wheat gluten and soy protein isolate, and established that their POP inhibitory potency at the concentration of 5 mg/mL was in the range of 58–72% and 56–79%, respectively, depending on the protease applied.

A hydrolyzate of corn gluten meal with 1% pepsin and its in vitro digesta exhibited POP inhibitory and antioxidant properties i.e. radical scavenging and ferric-reducing power, upregulation of catalase expression, and cellular antioxidant activity in human neuroblastoma SH-SY5Y cells (Chanajon, Noisa, and Yongsawatdigul 2022).

According to Suwanangul et al. (2022), hydrolyzates of proteins from oilseed meal (sacha inchi) exhibited POP inhibitory activity with IC_{50} values ranging from 0.67 to 20 mg/mL depending on peptide size, with the fraction below 1 kDa exhibiting the highest inhibition.

In vivo studies

Martorell et al. (2013) investigated neuroprotective potential of "Barquillo" – a protein-rich cocoa by-product, its hydrolyzates and peptides. The authors reported that treatment of "Barquillo" with Termamyl 120 L and Alcalase 2.4 L increased its POP inhibitory activity from approx. 23 to 43% and improved its protective ability against neurotoxicity caused by βA-peptides in *Caenorhabditis elegans*. Several peptides originating from cocoa's 21 kDa seed protein were identified (see Table 3). Notably, the most promising peptide Asp-Asn-Tyr-Asp-Asn-Ser-Ala-Gly-Lys-Trp-Trp-Val-Thr was also identified in different commercial chocolate samples (Martorell et al. 2013).

According to Katayama et al. (2014), a diet supplemented with 7% (w/w) soy peptide mixture composed of 64% di- and tripeptides prevented cognitive impairment during a 26-week mice trial. Similarly, according to Maebuchi et al. (2013), a supplementation of di/tripeptide-rich soybean protein hydrolyzate at a dose of 8 g/day over 8 weeks improved the delayed and immediate memory scores substantially in patients with mild cognitive dysfunction. Mazza et al. (2017) assayed the effects of dietary choices on cognitive decline progression over 12 months in Italian elderly individuals. According to the authors, diets including plant-based proteins and legumes improved the participants' cognitive performance. However, no systematic reviews were published on the impact of dietary plant protein-derived peptides on cognitive functions. The examples of food protein-derived peptides/hydrolyzates exhibiting procognitive properties in vivo are given in Table 4.

Bioavailability of POP inhibitory peptides

One of the major concerns influencing the physiological effects of bioactive peptides is their limited bioavailability. It is reflected in differences between in vitro and in vivo studies regarding their biological effects and can be caused by interactions with food matrix and GI tract components such as mucin or proteases in particular. Orally administrated peptides are vulnerable to proteolytic degradation in the stomach, intestine, brush border, and plasma. Generally, short peptides with Pro, Arg, or Trp residues at C-terminus exhibit high protease resistance (Udenigwe et al. 2021).

Table 4. Food protein-derived peptides exhibiting procognitive activity in vivo.

Peptide/hydrolyzate	Organism	Procognitive effect	Reference
Colostrinin™ (complex of 0.5-3 kDa peptides containing 20% Pro residues)	Rat	Increase of ability to concentrate	(Stańczykiewicz et al. 2017)
Colostrinin™ (complex of 0.5-3 kDa peptides containing 20% Pro residues)	Human	Improvement of cognitive sympoms and daily function during moderate AD	(Bilikiewicz and Gaus 2004)
Ser-Val-Asp-Gly-Lys-Glu-Asp-Leu-Ile-Trp and Ac-Arg-Lys-Trp-His-Phe-Leu-Trp-NH_2	*Caenorhabditis elegans*	Inhibition of βA oligomerization and protection against βA peptide-induced toxicty	(Manzanares et al. 2018)
Casein hydrolyzate containing 1.4 mg Val-Pro-Pro and 2 mg Ile-Pro-Pro	Human	Increase of cerebral blood flow velocity	(Akazawa et al. 2018)
Casein hydrolyzate containing 1.4 mg Val-Pro-Pro and 2 mg Ile-Pro-Pro	Human	Improvement of brain neural activation related to enhanced cognitive function and decrease of arterial stiffness	(Hamasaki et al. 2019)
Porcine brain hydrolyzate	Rat	Protection against βA peptide-induced toxicity, improvement of performance on reference, spatial and working memory tests	(Y.-T. Liu et al. 2019)
Chum salmon skin collagen hydrolyzate containing 86% oligopeptides	Mouse	Improvement of spatial memory and learning, reduction of oxidative stress in brain and number of apoptotic neurons	(Pei et al. 2010)
Porcine collagen hydrolyzate with average molecular weight of 1.2 kDa	Human	Induction of changes in the brain and improvement of language cognitive functions	(Koizumi et al. 2019)
Asp-Asn-Tyr-Asp-Asn-Ser-Ala-Gl y-Lys-Trp-Trp-Val-Thr	*Caenorhabditis elegans*	Protection against oxidative damage and βA peptide-induced toxicty	(Martorell et al. 2013)
Soy protein hydrolyzate containing 64% di- and tripeptides	Mouse	Prevention of cognitive impairment	(Katayama et al. 2014)
Soybean protein hydrolyzate rich in di/ tripeptides	Human	Improvement of delayed and immediate memory scores, increased dopamine serum level	Maebuchi et al. (2013)

Peptides that survive the GI transit and reach the gut can either exhibit their bioactivity there or can be absorbed into the bloodstream from which they reach their molecular targets (Xu et al. 2019). The transport of peptides across the intestinal epithelial cell monolayer occurs through either single or multiple mechanisms, depending on the peptides' properties e.g. molecular weight, amino acid composition, hydrophobicity, charge, and side chain flexibility (Xu et al. 2019). Di- and tripeptides are absorbed mostly via the PepT1 transporter present on the apical side of the gut epithelium. Larger peptides pass the intestinal barrier through paracellular transport via tight junctions, transcytosis via vesicles, and/or passive transcellular diffusion (Sato 2021). Although the absorbability of most peptides does not exceed 1%, some of them can reach the blood intact at concentrations within the micromolar range and remain bioactive in the plasma for several minutes to hours (Xu et al. 2019).

An additional difficulty in the search for effective POP inhibitors is that they must have the ability to penetrate the blood-brain barrier (BBB) – selective, semipermeable tissue formed by endothelial cells, serving as a regulatory interface between the blood and the CNS. It is one of the most challenging obstacles for molecules designed to be therapeutics for the CNS-related disorders, where numerous potential drug candidates fail (Banks 2015). The first food-derived POP inhibitory peptide whose ability to cross the BBB was confirmed was Pro-Pro-Leu (Hayes et al. 2016), previously generated from meat proteins using *in silico* analysis (Lafarga, O'Connor, and Hayes 2015). Several other small peptides capable of penetrating the brain were also discovered, including Pro-Gly, Pro-Hyp, Tyr-Pro, Trp-Tyr, Met-Lys-Pro, and Gly-N-methylated-Gly (Gly-Sar), although only the first of these is known to exhibit POP inhibitory properties (Ashmarin et al. 1998; Matsui, Yoshino, and Tanaka 2020). The transportability of peptides across the BBB is largely understudied, however, the known examples share a few common features such as small size up to several amino acid residues and high protease resistance (Matsui, Yoshino, and Tanaka 2020). The peptide sequence is also important, since Pro-Tyr, a sequence-reversed analogue of the brain transportable Tyr-Pro was not uptaken (M. Tanaka et al. 2019; Matsui, Yoshino, and Tanaka 2020).

Summary and future perspectives

The use of bioactive peptides from food proteins appears to be a very promising strategy in the prevention of various diseases. The biopeptides often have a similar mechanism of action to commonly prescribed synthetic pharmaceuticals, but unlike the latter, they are usually completely side-effect-free, although the possibility of adverse allergic reactions should not be ignored.

Numerous examples of biopeptides have been discovered, varying in origin, structure, and type of bioactivity, although the number of known POP inhibitory peptides is, comparatively, low and their potential health effects are largely unexplored. To the best of our knowledge, the majority of studies on POP repressing peptides have been done in vitro and none of the human studies, nor systematic reviews in which they were mentioned, was specifically designed to investigate the relationship between POP inhibitory properties of the peptides and their ability to influence the cognitive functions.

The difficulty in carrying out and interpreting the results of studies aimed to assess the neuroprotective potential of POP inhibitory peptides from foods is influenced by several factors. First of all, numerous peptides present nonspecific, multifunctional pharmacological properties. The IC_{50} values for the same inhibitor may differ depending on whether they were determined for the POP of bacterial, animal, or human origin. Moreover, some inhibitor-sensitive

biochemical processes that are controlled by POP are not dependent on its proteolytic activity. Lastly, most of the research concerning peptidic POP inhibitors has been performed on relatively pure proteins/hydrolyzates/peptides. The actual dietary sources of these peptides are often consumed while being a part of complex food matrices, comprising a myriad of CNS-affecting compounds capable of interacting and changing each other's properties in a manner that is often difficult to predict.

Several suggestions on the direction of future research concerning the POP inhibitory peptides can be made. Firstly, the efforts should be focused on optimizing the process of protein hydrolyzis in terms of obtaining very short peptides, as only these have been shown to be transportable beyond the BBB and potentially capable of exerting physiologically relevant effects. At the same time, longer peptides should not be excluded from consideration, since they can act as pro-drugs, from which shorter, more bioavailable peptides can be released following continued hydrolyzis by endogenous enzymes. On the other hand, hydrolyzates characterized by very high hydrolyzis degrees may contain mostly free amino acids instead of peptides, which do not exhibit POP inhibitory properties. Thus, the simulated GI digestion of the peptides in question should be applied whenever possible, to assess whether their inhibitory potency and potential bioavailability would be affected. Secondly, the search for POP inhibitory peptides should not be narrowed to Pro-rich proteins only, as Pro-free peptides also exhibited notable repressive properties in vitro. Instead, proteins rich in hydrophobic amino acid residues should be investigated more extensively.

In summary, although some milk, meat, fish, and plant protein-derived peptides have the potential to be applied as natural, procognitive nutraceuticals, their effectiveness requires further evaluation, especially in clinical trials. We demonstrated that the important features of the most promising POP-inhibiting peptides are very short sequence, high content of hydrophobic amino acids, and usually the presence of Pro residue (Table 5).

Table 5. List of abbreviations.

Abbreviation	Explanation
αS	α-synuclein
βA	β-amyloid
ACE	angiotensin-converting enzyme
AD	Alzheimer's disease
ADHD	attention deficit hyperactivity disorder
BBB	blood-brain barrier
CNS	central nervous system
DPP IV	dipeptidyl peptidase IV
GAP-43	growth-associated protein 43
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GI	gastrointestinal
IC_{50}	half-maximal inhibitory concentration
IP3	inositol 1,4,5-triphosphate
NMDA	N-methyl-D-aspartic acid
POP	prolyl oligopeptidase
PP2A	protein phosphatase 2 A
PPI	protein-protein interaction
PSP	proline-specific peptidase
ROS	reactive oxygen species
VPA	valproic acid

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No potential conflict of interest was reported by the authors.

Author contributions

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