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Prolyl Endopeptidase Inhibitory Activity of Unsaturated Fatty Acids

YOON-SEOK PARK, HYUN-JUNG JANG, KYUNG-HO LEE, TAE-RYONG HAHN, AND YOUNG-SOOK PAIK*

College of Environment and Applied Chemistry and Plant Metabolism Research Center, Kyung Hee University, Suwon 449-701, Korea

Prolyl endopeptidase (PEP, EC 3.4.21.26) is widely distributed in various organs, particularly in the brains of amnestic patients. Evaluation of PEP levels in postmortem brains of Alzheimer's disease patients revealed significant increases in PEP activity, suggesting that a specific PEP inhibitor can be a good candidate for an antiamnestic drug. In this study, mono- and polyunsaturated fatty acids were investigated to determine their role as PEP inhibitors. Oleic, linoleic, and arachidonic acids, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) showed PEP inhibitory activities (IC_{50} values of 23.6 ± 0.4, 43.8 ± 1.8, 53.4 ± 1.2, 99.4 ± 1.2, and 46.2 ± 1.0 μM, respectively), indicating that they were effective PEP inhibitors, with inhibition constant (K_i) values of 26.7 ± 0.3, 51.0 ± 0.7, 91.3 ± 3.1, 247.5 ± 2.6, and 89.0 ± 2.3 μM, respectively. Oleic acid showed the highest PEP inhibitory activity. Dixon plots of PEP inhibition showed oleic, linoleic, and arachidonic acids, EPA, and DHA are noncompetitive inhibitors; despite higher IC_{50} values of these unsaturated fatty acids than strong natural inhibitors, they may have potential use in preventing memory loss.

KEYWORDS: Prolyl endopeptidase inhibitor; antiamnestic; oleic acid; linoleic acid; arachidonic acid; DHA; EPA

INTRODUCTION

Mental illness in elderly people has been found to be associated with poor diet. In particular, deficiency of micronutrients such as vitamins B_1, B_2, B_6, B_{12}, and folate, frequently described in elderly people, is associated with cognitive impairment (*1*). Studies showed that a diet rich in antioxidants such as vitamins C and E and polyphenols can help reduce the risk of dementia by eliminating harmful free radicals and decreasing the level of oxidative stress (*2*, *3*). Although few data are available on the role of macronutrient intake in age-related cognitive decline, several findings suggest that saturated and unsaturated fatty acids also affect age-related memory loss in elderly people; diets rich in meat and dairy products, e.g., diets high in saturated fatty acid and cholesterol, increased the risk of dementia (*4*), whereas the reverse was observed with mono- and polyunsaturated fatty acid intakes (*5*, *6*). Similarly, Morris et al. (*7*) reported that a high intake of monounsaturated fat and a high ratio of polyunsaturated to saturated fat intake were inversely related to the risk of cognitive decline among elderly people.

The Mediterranean diet pattern practiced by an elderly population in southern Italy, which is based on complex carbohydrates, fiber, and nonanimal fat, appears to protect against age-related cognitive decline (*5*). The mean consumption of olive oil (46 g/day) in the diet was particularly high, with a clear reduction of the age-related cognitive decline risk found only in population samples with a high intake of olive oil (>100 g/day) (*8*). This protective effect is attributed to the high oleic acid content of olive oil. ω-3 Polyunsaturated fatty acids in fatty fish such as salmon, tuna, and mackerel were also found to protect the brain against mild deterioration in memory or intellectual functioning associated with the aging process (*7*, *9*); intake of marine ω-3 polyunsaturated fatty acids such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) reduced the risk of the impairment of cognitive function and speed (*6*), suggesting that mono- and polyunsaturated fatty acids such as oleic acid, DHA, and EPA play important roles in the protection of age-related cognitive decline.

Prolyl endopeptidase (PEP, EC 3.4.21.26, also referred to as prolyl oligopeptidase), widely distributed in various organs, particularly in the human brain, is classified as a serine protease based on its inhibition by diisofluorophosphate and to a lesser extent by phenylmethylsulfonylfluoride (*10*). PEP cleaves peptide bonds on the carboxylic sides of prolyl residue within the polypeptides of less than 30 amino acids such as vasopressin, substance P, and thyrotropin-releasing hormone (*10*−*13*). Evaluation of the PEP levels in postmortem brains obtained from Alzheimer's disease patients revealed significant increases in the enzyme activity, suggesting that PEP plays a functional role in amyloidgenesis in the brain (*10*, *14*). Some natural and synthetic PEP inhibitors showed dose-dependent cognition-

* To whom correspondence should be addressed. Tel: 82 31 201 2422. Fax: 82 31 202 7337. E-mail: paikys@khu.ac.kr.

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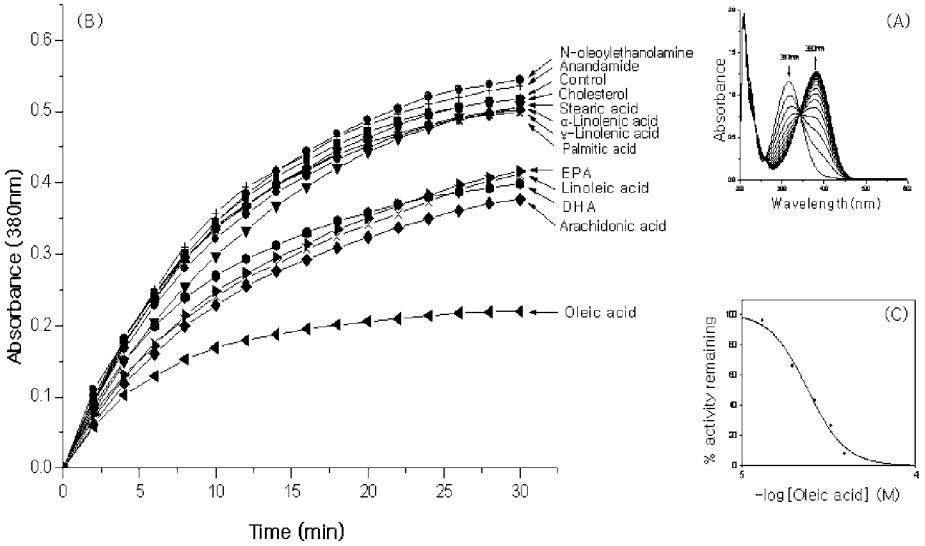

Figure 1. (**A**) UV–vis spectra for the production of *p*-nitroaniline. *p*-Nitroaniline was produced by reacting Z-Gly-Pro-*p*NA with PEP in 0.1 M phosphate buffer (pH 7.0, 37 °C, and 10 min scanning interval). (**B**) Typical inhibition pattern of *p*-nitroaniline produced from the reaction of Z-Gly-Pro-*p*NA with PEP in 0.1 M phosphate buffer (pH 7.0, 37 °C, and 2 min scanning interval) at 380 nm in the absence and presence of saturated fatty acid, sodium salts of unsaturated fatty acid, and other compounds (8 μg/mL). (**C**) Plots of % activity remaining from the reaction of Z-Gly-Pro-*p*NA with PEP vs –log (oleic acid). Each data point represents the average of triplicate measurements.

enhancing activities in rats with scopolamine-induced amnesia (*10*). Thus, a specific PEP inhibitor can be a good candidate for antiamnestic drugs for curing memory loss and neuropathological disorders.

Several synthetic PEP inhibitors (Ono-1603, JTP-4819, and S-17092-1) have entered clinical trials as cognitive enhancers (*15*). S-17092 as revealed by pharmacodynamic and pharmacokinetic studies showed a potent dose-dependent inhibitory effect on plasmatic PEP and improved performance in two verbal memory tests in elderly healthy persons, indicating that S-17092 is suitable for once-daily dosing without causing any serious adverse side effects (*15*).

At present, drug treatments for Alzheimer's disease are primarily focused on treating the symptoms rather than preventing mental deterioration. Therefore, the objective of our work was to search dietary sources for their potential health benefits associated with age-related memory loss. Because their deficiencies have been linked with decreased memory loss and mental abilities (*16*, *17*), mono- and polyunsaturated fatty acids, abundantly found in vegetable seeds and fatty fish, were studied for the effects on PEP activity.

MATERIALS AND METHODS

Materials. PEP (from *Flavobacterium meningosepticum*) and its substrate, benzyloxycarbonyl-glycyl-L-prolyl-*p*-nitroanilide (Z-Gly-Pro-*p*NA), were purchased from Seikagaku Co. (Tokyo, Japan). Benzyloxycarbonyl-L-prolyl-prolinal (Z-Pro-prolinal) as a positive control was purchased from Biomol Research Laboratories Inc. (Philadelphia, PA). All chemicals including sodium salts of oleic, linoleic, and arachidonic acids, EPA, DHA, and solvents, either reagent or high-performance liquid chromatography grade, were obtained from Sigma-Aldrich Co. (St. Louis, MO).

Synthesis of N-Oleoylethanolamine. Oleic acid (50 mg, 0.18 mmol), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide HCl (41 mg, 0.21 mmol), and 1-hydroxybenzotriazole hydrate (29 mg, 0.21 mmol) were dissolved in 4 mL of CH_2Cl_2 and stirred for 10 min, followed by the addition of ethanolamine (13 mg, 0.21 mmol) and triethylamine (54 mg, 0.53 mmol). The reaction mixture was stirred for 15 min at room temperature and added to a solution of 10 mL of CH_2Cl_2 and 10 mL of H_2O. The organic layer was successively washed with saturated aqueous citric acid, H_2O, saturated $NaHCO_3$, and finally saturated brine. The solution was treated with Na_2SO_4 anhydrous, and the solvent was evaporated. Preparative thin-layer chromatography ($CHCl_3$:MeOH = 10:1) gave 29 mg of N-oleylethanolamine (R_f 0.56 $CHCl_3$:MeOH = 5:1). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.02 (1H, br s, NH), 5.31 (2H, m, H-9, 10), 3.69 (2H, t, J = 4.8 Hz, H-2′), 3.40 (2H, m, H-1′), 2.17 (2H, t, J = 7.2 Hz, H-2), 1.97 (4H, m, H-8, 11), 1.60 (2H, m, H-3), 1.2−1.3 (20H, H-4−7,12−17), 0.85 (3H, t, J = 6.8, H-18). ^{13}C NMR (100 MHz, $CDCl_3$): δ 174.37 (C-1), 129.91, 129.62 (C-9, 10), 62.53 (C-2′), 42.48 (C-1′), 36.71 (C-2), 31.94, 29.81, 29.76, 29.57, 29.37, 29.37, 29.31, 29.31, 29.18 (C-4−7, 12−16), 27.27, 27.23 (C-8, 11), 25.77 (C-3), 22.74 (C-17), 14.18 (C-18). The assignments were based on HSQC, HMBC, and ^{1}H−^{1}H COSY experiments.

Table 1. IC_{50} and K_i Values of Mono- and Polyunsaturated Fatty Acids

compound	IC_{50} (μM)[a]	K_i (μM)[a]
oleic acid	23.6 ± 0.4	26.7 ± 0.3
linoleic acid	43.8 ± 1.8	51.0 ± 0.7
arachidonic acid	53.4 ± 1.2	91.3 ± 3.1
EPA	99.4 ± 1.2	247.5 ± 2.6
DHA	46.2 ± 1.0	89.0 ± 2.3

[a] Data are means ± SE of three separate experiments.

PEP Inhibition Assay. UV−vis spectra were recorded on Varian Cary 300 and Jasco V-530 spectrophotometers. The PEP activity was assayed using the method of Yoshimoto et al. with minor modifications (*18*−*20*). A mixture of 800 μL of 0.1 M phosphate buffer (pH 7.0), 80 μL of 2 mM Z-Gly-Pro-*p*NA in 40% 1,4-dioxane, and 40 μL of sample solution (sodium salts of fatty acids; 1 mg/mL MeOH stock solution diluted with 0.1 M phosphate buffer, pH 7.0) was preincubated at 37 °C for 10 min. The reaction was started by adding 80 μL of 0.1 unit/mL PEP in 0.1 M phosphate buffer (pH 7.0) at 37 °C. After incubation for 30 min, the amount of released *p*-nitroaniline of the solution (A) was determined colorimetrically based on the absorbance at 380 nm. The A_{380} value of the mixture containing 960 μL of 0.1 M phosphate buffer (pH 7.0) and 40 μL of sample solution was separately measured as mentioned above (B). A control was made by adding 40 μL of 0.1 M phosphate buffer instead of 40 μL of the sample solution used in A. The percentage of inhibition was calculated using the following equation: percentage of inhibition = [{A_{380} of control − (A − B)}/A_{380} of control] × 100. Triplicate samples were analyzed, and IC_{50} values were determined graphically based on the curves of the enzyme activity vs inhibitor concentration.

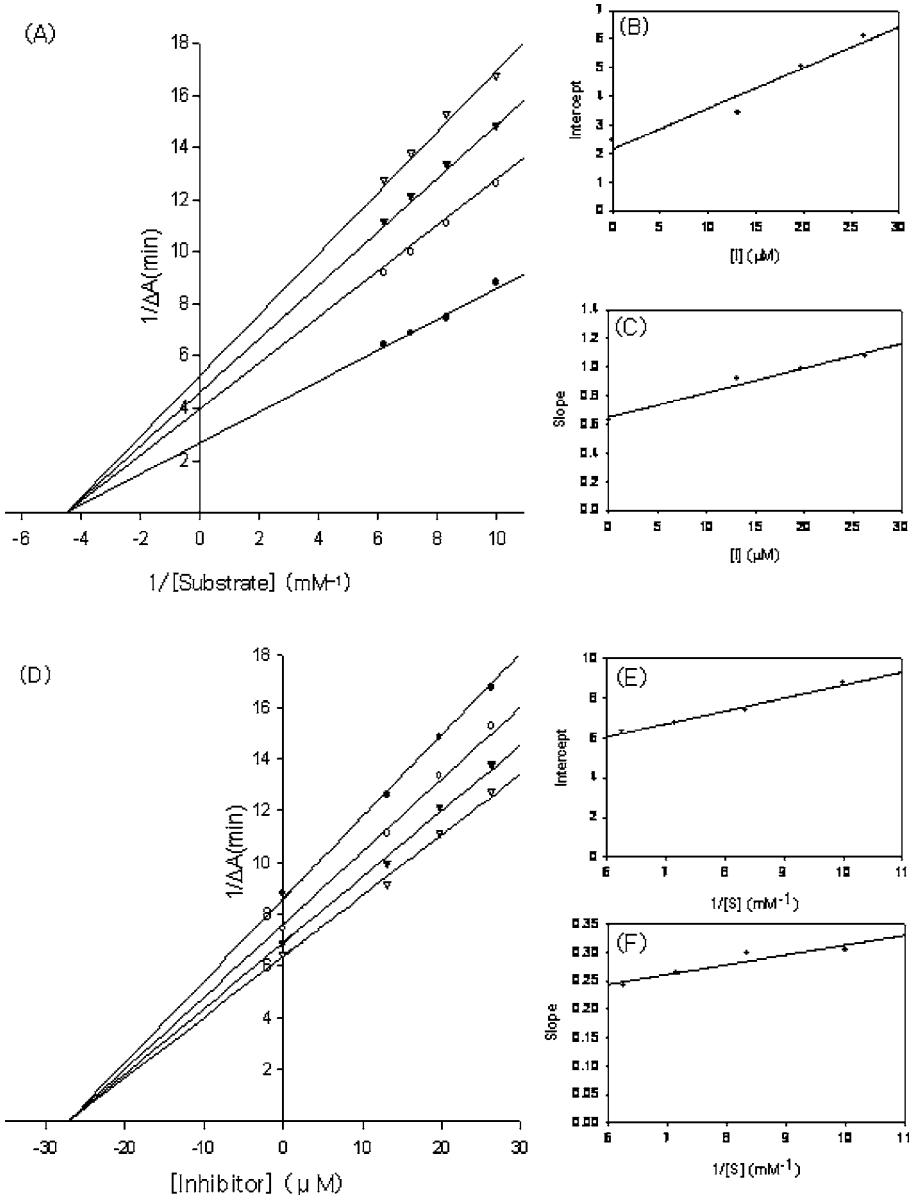

Figure 2. (**A**) Lineweaver–Burk plots of PEP inhibition by oleic acid [in the absence (●) and presence of 13.1 (○), 19.7 (▼), and 26.3 (▽) μM oleic acid]. (**B**) Secondary plot of intercepts (*i*) taken from Lineweaver–Burk plots vs oleic acid concentration. (**C**) Secondary plot of slopes (*s*) taken from Lineweaver–Burk plots vs oleic acid concentration. (**D**) Dixon plots of PEP inhibition by oleic acid. [S] = 0.1 (●), 0.12 (○), 0.14 (▼), and 0.16 mM (▽). (**E**) Secondary plot of intercepts (*i*) taken from Dixon plots vs reciprocal of the Z-Gly-Pro-*p*NA concentration. (**F**) Secondary plots of slopes (*s*) taken from Dixon plots vs reciprocal of the Z-Gly-Pro-*p*NA concentration.

Statistics. Intergroup comparisons of data were made using Enzyme Kinetics Module (Add-on software for SigmaPlot 2000) from SPSS Science (San Francisco, CA).

RESULTS AND DISCUSSION

The role of dietary fat in dementia is arousing much interest (*9*). Studies have found that DHA deficiency can have marked consequences, including retarded visual acuity, cognitive impairment, cerebellar dysfunction, and various other neurological disorders (*16*). Symptoms of Alzheimer's disease, such as short-term memory loss, depressed mood, and inability to sleep (symptoms often found in the elderly), are markedly ameliorated by treatment with an essential fatty acid preparation (SR-3) containing ω-3 (*21*). Among all human organs (excluding the adipose tissue), the nervous system has the highest lipid content. An adult brain is composed of 50−60% lipid on a dry weight basis, 35% of which is accounted for by polyunsaturated fatty acids (*16*, *22*). The fatty acid composition of the neuronal cell membrane phospholipids reflects their intake in the diet; the more kinked the unsaturated fatty acid is, the more space it will take up when used as a building block of the cell membrane phospholipids, thereby increasing the fluidity and, probably, the functionality of the cell membrane (*16*).

Using oleic, linoleic, α-linolenic, γ-linolenic, and arachidonic acids, EPA, and DHA, the relationships between mono- and polyunsaturated fatty acids and the inhibitory activity of PEP, which has been suggested to participate in the learning and memory processes, as well as the effects of saturated fatty acids (palmitic and stearic acids) and cholesterol on the activity, were evaluated. Synthesized N-oleoylethanolamine (oleic acid residue joined to ethanolamine through an amide link; see Materials and Methods section) and commercial anandamide (arachidonic acid residue joined to ethanolamine through an amide link) were used to examine the possibility of carboxyl functional group in

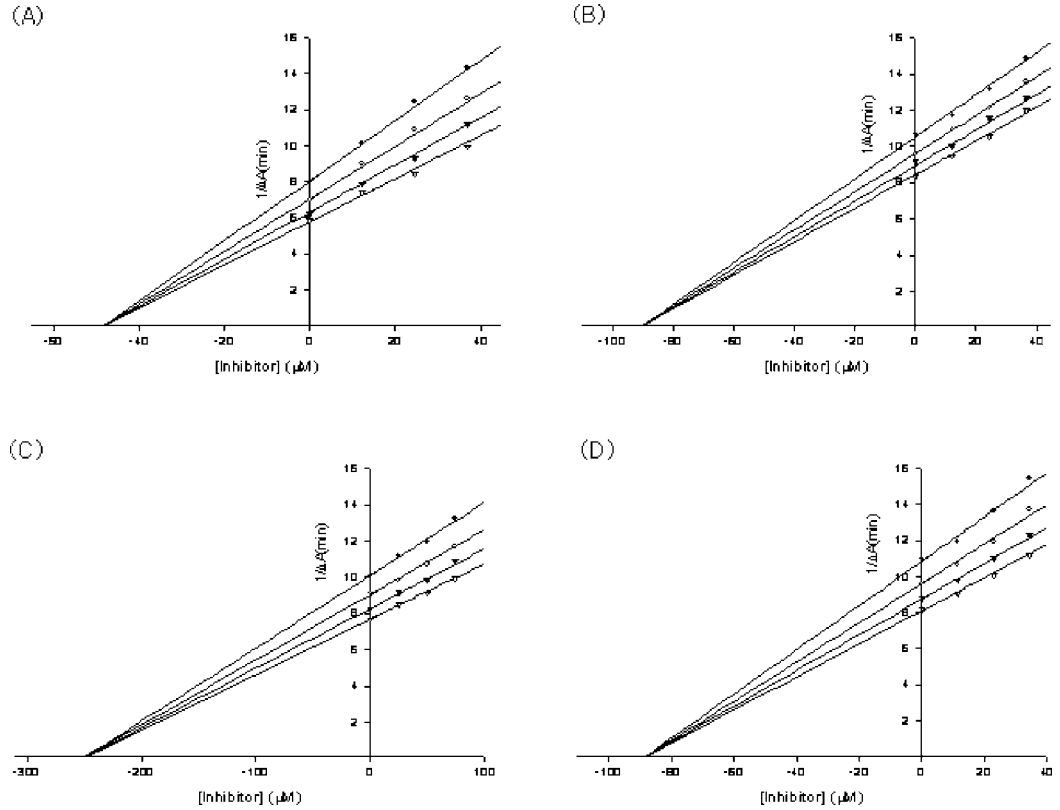

Figure 3. (**A**) Dixon plots of PEP inhibition by linoleic acid, (**B**) arachidonic acid, (**C**) EPA, and (**D**) DHA. [S] = 0.1 (●), 0.12 (○), 0.14 (▼), and 0.16 mM (▽).

oleic and arachidonic acids playing a crucial role in the PEP inhibitory activity.

Using Z-Gly-Pro-*p*NA as a substrate for the measurement of PEP inhibitory activity, the amount of released *p*-nitroaniline was determined colorimetrically at 380 nm. **Figure 1A** shows the UV−vis spectra for the formation of *p*-nitroaniline from the reaction of Z-Gly-Pro-*p*NA with PEP at 10 min scanning intervals. The λ_{max} of Z-Gly-Pro-*p*NA at 317 nm decreased, whereas that of *p*-nitroaniline at 380 nm increased. Z-Pro-prolinal, a synthetic PEP inhibitor, was used as a reference compound of the positive control (IC_{50} = 2.19 nM) (*19*).

Upon preliminary examination of saturated and unsaturated fatty acids at 8 μg/mL for the inhibition of PEP, palmitic and stearic acids showed little activities, whereas oleic acid showed 52.2 ± 1.3% inhibition (**Figure 1B**). Because high concentration and high molecular weight of fatty acids resulted in solubility problems, the sodium salt of oleic acid, which also showed PEP inhibitory activity (57.1 ± 1.6%) at a similar level to that of oleic acid, was examined. Sodium salts of linoleic acid, arachidonic acid, EPA, and DHA (8 μg/mL each) showed 15.9 ± 0.3, 19.5 ± 2.0, 15.8 ± 0.3, and 15.5 ± 0.7% PEP inhibitory activities, respectively (**Figure 1B**), whereas cholesterol, α- and γ-linolenic acids (both forms of free acids and sodium salts), N-oleoylethanolamine, and anandamide showed no inhibitory activity (**Figure 1B**). Five unsaturated fatty acids (oleic, linoleic, arachidonic acids, EPA, and DHA) showing more than 10% PEP inhibition at 8 μg/mL (**Figure 1B**) were further investigated at various concentrations to determine their IC_{50} values. All acids showed dose-dependent PEP inhibitory activities, with oleic acid showing the most effective activity with an IC_{50} value of 23.6 ± 0.4 μM; IC_{50} values of linoleic acid, arachidonic acid, EPA, and DHA were 43.8 ± 1.8, 53.4 ± 1.2, 99.4 ± 1.2, and 46.2 ± 1.0 μM, respectively (**Figure 1C** and **Table 1**). Although statistically significant, changes in the levels of PEP inhibitory activity of oleic acid and other unsaturated fatty acids were relatively low.

Lineweaver−Burk plots of the PEP inhibition by oleic acid indicate that oleic acid is a noncompetitive inhibitor (**Figure 2A**). The secondary Lineweaver−Burk plots show a linear relationship for both intercepts (*i*) vs oleic acid concentration (**Figure 2B**) and slope (*s*) vs oleic acid concentration (**Figure 2C**). Dixon plots of the PEP inhibition by oleic acid also indicate that oleic acid is a noncompetitive inhibitor with an inhibition constant (K_i) value of 26.7 μM (**Figure 2D** and **Table 1**). The secondary Dixon plots show linear relationships between intercepts (*i*) vs reciprocal of the Z-Gly-Pro-*p*NA concentration and slope (*s*) vs reciprocal of the Z-Gly-Pro-*p*NA concentration (**Figure 2E**,**F**, respectively). **Figure 3A**−**D**, which shows Dixon plots of the PEP inhibition by linoleic acid, arachidonic acid, EPA, and DHA, respectively, indicate that all examined unsaturated fatty acids are noncompetitive inhibitors. The inhibition constant (K_i) values of linoleic acid, arachidonic acid, EPA, and DHA were 51.0 ± 0.7, 91.3 ± 3.1, 247.5 ± 2.6, and 89.0 ± 2.3 μM, respectively (**Table 1**).

Oleic acid showed the most effective PEP inhibition (**Figure 1B**), whereas saturated fatty acids (palmitic and stearic acids) showed little inhibitory activity, which suggests that the double bond in oleic acid (18:1, Δ^9) could play an important role in PEP inhibition. Linoleic acid (18:2, $\Delta^{9,12}$), arachidonic acid (20:4, $\Delta^{5,8,11,14}$), EPA (20:5, $\Delta^{5,8,11,14,17}$), and DHA (22:6, $\Delta^{4,7,10,13,16,19}$) showed lower PEP inhibition than oleic acid, and α-linolenic acid (18:3, $\Delta^{9,12,15}$) and γ-linolenic acid (18:3, $\Delta^{6,9,12}$), both forms of free acids and sodium salts, showed little PEP inhibition. These results suggest that the position and number of double bonds in the unsaturated fatty acids are responsible for the inhibitory activity, although the exact mechanism involved remains to be elucidated. N-oleoylethanolamine, synthesized from oleic acid and ethanolamine to prepare the

amide bond, showed little PEP inhibitory activity (**Figure 1B**), suggesting that the carboxyl functional group in oleic acid also could play a crucial role in the PEP inhibition. Anandamide also showed no PEP inhibitory activity (**Figure 1B**), further indication that carboxyl functional group in arachidonic acid could be critical in the PEP inhibition. Our previous report on two ginkgolic acids from *Ginkgo biloba* also suggested that carboxyl functional group is important in the PEP inhibition (*19*). These results suggest that not only the position of the double bond but also the presence of carboxyl group in the unsaturated fatty acids is responsible for PEP inhibition.

Although IC_{50} values of oleic, linoleic, and arachidonic acids, EPA, and DHA (23.6−99.4 μM) were higher than those reported for strong natural inhibitors such as isotamaraxin (IC_{50}, 0.25 μM) (*23*), ginkgolic acid (IC_{50}, 0.62 μM) (*19*), staurosporine (IC_{50}, 0.77 μM) (*24*), and kinapsin-24 (IC_{50}, 1.14 μM) (*25*), they may have potential use in the prevention of memory loss. Diets high in saturated fatty acids and cholesterol increased the risk of dementia (*4*). On the other hand, an inverse relationship was found between mono- and polyunsaturated fatty acid energy intakes and age-related cognitive decline (*5*, *6*).

In summary, oleic acid showed the most effective PEP inhibition with an IC_{50} value of 23.6 ± 0.4 μM and a K_i value of 26.7 ± 0.3 μM. Linoleic acid, DHA, and arachidonic acid with respective IC_{50} values of 43.8, 46.2, and 53.4 μM also showed significant PEP inhibition. Increasing evidence shows that a correct balance between ω-3 and ω-6 fatty acids in the brain cell membranes is important for the maintenance of mental health, and higher dosages of ω-3 fatty acid (2−4 g daily) may ameliorate the symptoms of several psychiatric conditions (*16*). The current western diet does not supply ω-3 and ω-6 fatty acids in the desired proportion of 1:4. Thus, a lack of the proper balance of essential fatty acids, linoleic (ω-6) and linolenic (ω-3) acids, may be playing a role in the dementia.

A high intake of monounsaturated fat and a high ratio of polyunsaturated to saturated fat intake are inversely related to the risk of cognitive decline (*7*). Therefore, consumption of oleic acid rich in a typical Mediterranean diet, linoleic and linolenic acids rich in vegetable seeds, and DHA and EPA rich in a fatty fish diet is highly recommended to protect against mild deterioration in memory and intellectual functioning.

LITERATURE CITED

(1) Calvaresi, E.; Bryan, J. B Vitamins, cognition, and aging: A review. *J. Gerontol.* **2001**, *56B*, 327−339.

(2) Engelhart, M. J.; Geerlings, M. I.; Ruitenberg, A.; van Swieten J. C.; Hofman, A.; Witteman, J. C.; Breteler, M. M. Dietary intake of antioxidants and risk of Alzheimer disease. *J. Am. Med. Assoc.* **2002**, *287*, 3223−3229.

(3) Markesbery, W. R. Oxidative stress hypothesis in Alzheimer's disease. *Free Radical Biol. Med.* **1997**, *23*, 134−147.

(4) Morris, M. C.; Evans, D. A.; Bienias, J. L.; Tangney, C. C.; Bennett, D. A.; Aggarwal, N.; Schneider, J.; Wilson, R. S. Dietary fats and the risk of incident Alzheimer disease. *Arch. Neurol.* **2003**, *60*, 194−200.

(5) Solfrizzi, V.; Panza, F.; Torres, F.; Mastroianni, F.; Del Parigi, A.; Venezia, A.; Capurso, A. High monounsaturated fatty acids intake protects against age-related cognitive decline. *Neurology* **1999**, *52*, 1563−1569.

(6) Kalmijin, S.; van Boxtel, M. P. J.; Ocke, M.; Verschuren, W. M. M.; Kromhout, D.; Launer, L. J. Dietary intake of fatty acids and fish in relation to cognitive performance at middle age. *Neurology* **2004**, *62*, 275−280.

(7) Morris, M. C.; Evans, D. A.; Bienias, J. L.; Tangney, C. C.; Wilson, R. S. Dietary fat intake and 6-year cognitive change in an older biracial community population. *Neurology* **2004**, *62*, 1573−1579.

(8) Panza, F.; Solfrizzi, V.; Colacicco, A. M.; D'Introno, A.; Capurso, C.; Torres, F.; Del Parigi, A.; Capurso, S.; Capurso, A. Mediterranean diet and cognitive decline. *Public Health Nutr.* **2004**, *7*, 959−963.

(9) Barberger-Gateau, P.; Letenneur, L.; Deschamps, V.; Peres, K.; Dartigues, J.-F.; Renaud, S. Fish, meat, and risk of dementia: Cohort study. *B. M. J.* **2002**, *325*, 932−933.

(10) Cunningham, D. F.; O'Connor, B. Proline specific peptidase. *Biochim. Biophys. Acta* **1997**, *1343*, 160−186.

(11) Walter, R.; Shlank, H.; Glass, J. D.; Schwartz, I. L.; Kerenyi, T. D. Leucylglycinamide released from oxytocin by human uterine enzyme. *Science* **1971**, *173*, 827−829.

(12) Kalwant, S.; Porter, A. G. Purification and characterization of human brain prolyl endopeptidase. *Biochem. J.* **1991**, *276*, 237−244.

(13) Odaka, C.; Mizuochi, T.; Shirasawa, T.; Morain, P.; Checler, F. Murine T cells expressing high activity of prolyl endopeptidase are susceptible to activation-induced cell death. *FEBS Lett.* **2002**, *512*, 163−167.

(14) Aoyagi, T.; Wada, T.; Nagai, M.; Kojima, F.; Harada, S.; Takeuchi, T.; Takahashi, H.; Hirokawa, K.; Tsumita, T. Deficiency of kallikrein-like enzyme activities in cerebral tissue of patients with Alzheimer's disease. *Experientia* **1991**, *46*, 94−97.

(15) Polgar, L. Structure−function of prolyl ologopeptidase and its role in neurological disorders. *Curr. Med. Chem.* **2002**, *2*, 251−257.

(16) Haag, M. Essential fatty acids and the brain. *Can. J. Psychiatry* **2003**, *48*, 195−203.

(17) McCann, J. C.; Ames, B. N. Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. *Am. J. Clin. Nutr.* **2005**, *82*, 281−295.

(18) Yoshimoto, T.; Walter, R.; Tsuru, D. Proline-specific endopeptidase from *Flavobacterium*. *J. Biol. Chem.* **1980**, *255*, 4786−4792.

(19) Lee, J.-H.; Lee, S.-Y.; Lee, K.-S.; Jang, H.-J.; Lee, K.-H.; Hahn, T.-R.; Paik, Y.-S. Prolyl endopeptidase inhibitors from the leaves of *Ginkgo biloba*. *Planta Med.* **2004**, *70*, 1228−1230.

(20) Jang, H.-J.; Paik, Y.-S. Prolyl endopeptidase inhibitory activity of omega-3 polyunsaturated fatty acids. *Agric. Chem. Biotechnol.* **2004**, *47*, 178−180.

(21) Yehuda, S.; Rabinovitz, S.; Carraso, R. L.; Mostofsky, D. I. Essential fatty acid preparation (SR-3) improves Alzheimer's patients' quality of life. *Int. J. Neurosci.* **1996**, *87*, 141−149.

(22) Yehuda, S.; Rabinovitz, S.; Mostofsky, D. I. Essential fatty acids are mediators of brain biochemistry and cognitive functions. *J. Neurosci. Res.* **1999**, *56*, 565−570.

(23) Sultanova, N.; Makhmoor, T.; Yasin, A.; Abilov, Z. A.; Omurkamzinova, V. B.; Atta-ur-Rahman; Choudhary, M. I. Isotamarixen−A new antioxidant and prolyl endopeptidase-inhibiting triterpenoid from *Tamarix hispida*. *Planta Med.* **2004**, *70*, 65−67.

(24) Kimura, K.; Kawaguchi, N.; Yoshihama, M.; Kawanishi, G. Staurosporine, a prolyl endopeptidse inhibitor. *Agric. Biol. Chem.* **1990**, *54*, 3021−3022.

(25) Song, K.-S.; Raskin, I. A prolyl endopeptidase-inhibiting benzofuran dimer from *Polyozellus* multiflex. *J. Nat. Prod.* **2002**, *65*, 76−78.

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