ISOLATION AND CHARACTERIZATION OF OPIOID PEPTIDES DERIVED FROM WHEAT GLUTEN

S.-I. Fukudome and M. Yoshikawa*

Research Control Department, Nisshin Flour Milling Co., Ltd.,
Nihonbashi, Chuo-ku, Tokyo 103, Japan
* Department of Food Science and Technology, Faculty of Agriculture, Kyoto University,
Kyoto 606, Japan

Abstract

Five opioid peptides, Gly-Tyr-Tyr-Pro-Thr, Gly-Tyr-Tyr-Pro, Tyr-Gly-Gly-Trp-Leu, Tyr-Gly-Gly-Trp and Tyr-Pro-Ile-Ser-Leu were isolated from enzymatic digests of wheat gluten. These peptides were named gluten exorphins A5, A4, B5, B4 and C, respectively. The gluten exorphins A5/A4 sequences were found at 15 sites in the primary structure of the high molecular weight glutenin and were highly selective for δ-receptors. The structure-activity relationships of gluten exorphins A were characteristic in that their N-terminal Gly residues were important for the expression of their activities. Gluten exorphin B5, which corresponds to [Trp^4,Leu^5]enkephalin, showed the highest activity among the exogenous opioid peptides tested. Its IC_{50} values were 0.05 μM and 0.017 μM in the guinea pig ileum (GPI) and the mouse vas deferens (MVD) assays, respectively. Gluten exorphin C had a structure quite different from those of endogenous and exogenous opioid peptides so far reported in that the N-terminal Tyr residue was the only aromatic amino acid.

Introduction

An opioid-active material in a pepsin digest of wheat gluten was found by Zioudrou et al. in 1979. They recognized the activity in the digest in both assays, inhibition of the electrically stimulated contraction of MVD and the inhibition of adenylate cyclase of neuroblastoma X-glioma hybrid cells (23). Huebner et al. also recognized opioid activities in fragments derived from gliadin fractions by a radioreceptor assay (8). Graf et al. synthesized a peptide fragment of α-gliadin (Tyr-Pro-Gln-Pro-Gln-Pro-Phe), which is homologous to ß-casomorphin, but its opioid activity was very weak (6). On the other hand, it has been reported that the oral administration of wheat gluten digests influenced gastrointestinal motility or hormone release, and the effects were reversed by naloxone (11, 16, 17). These reports also suggested the existence of opioid peptides in the digests. However, their structure and character have not been reported yet. In this study, to obtain this information, we have attempted to isolate such opioid peptides from enzymatic digests of wheat gluten.

Correspondence should be directed to:
Dr. Masaaki Yoshikawa, Department of Food Science and Technology, Faculty of Agriculture, Kyoto University, Kyoto 606, Japan

Material and Methods

Substances

Wheat gluten was obtained from Goodman Fielder Mills Ltd. Pepsin, trypsin and chymotrypsin were from Sigma Chemical Co. Thermolysin was from Peptide Institute Inc. Naloxone was from U.S.P.C., Inc. [^{3}H][D-Ala^2, MePhe^4, Glyol^5] enkephalin), [^{3}H]DADLE ([^{3}H][D-Ala^2, D-Leu^5] enkephalin) and [^{3}H]EKC ([^{3}H]ethylketocyclazocine) were from Amersham. Other reagents used were reagent grade or better.

Enzymatic digestion of wheat gluten

Wheat gluten (50 mg/ml solution) was digested with pepsin (0.5 mg/ml) in 0.02N HCl (pH 2.0) or 17 hrs at 36°C. After the digestion, the pH of the solution was adjusted with 1 N NaOH to 7.0. Then, the solution was boiled and centrifuged. The supernatant was lyophilized. The peptic digest(50 mg/ml solution) was further digested with trypsin or chymotrypsin or thermolysin (0.5 mg/ml, respectively) in distilled water for 5 hrs at 36°C, boiled and centrifuged.

Purification of peptides

Separations of peptides in the digests were accomplished by reversed phase HPLC on an octadecyl silica (ODS) column (Cosmosil 5C$_{18}$-AR, 20 x 250 mm, Nacalai Tesque Inc.). An amount of 100 mg digest was applied to the column and was eluted with a linear gradient between 0 to 40% acetonitrile containing 0.05% trifluoroacetic acid (TFA) at a flow rate of 10 ml/min. The eluate was monitored at 230 nm. Individual fractions were dried with a centrifugal concentrator and their opioid activities were measured in the MVD assay. The opioid-active fractions were purified on an ODS column (Cosmosil 5C$_{18}$-AR, 4.6 x 150 mm), a phenyl silica column (Cosmosil 5Ph, 4.6 x 250 mm) and cyanopropyl silica column (Cosmosil 5CN-R, 4.6 x 250 mm) from Nacalai Tesque Inc. and a phenetyl silica column (Develosil PhA-5, 4.6 x 250 mm) from Nomura Chemical Inc.. The columns were developed by a linear gradient between 0 to 50% acetonitrile containing 0.05% TFA or 10mM potassium-sodium phosphate buffer (pH 7) at 1 ml/min. The eluates were monitored at 215 nm to 235 nm. The active fractions were purified on the same ODS column as used for the first step of purification.

Opioid activity assay

The measurements of opioid activities were performed by the MVD and GPI assays (4). For the MVD assay, the mouse vas deferens was suspended with 0.2 g tension in a magnus tube containing a Mg^{2+}-free Krebs-Ringer solution and stimulated (30 V, 1.2 msec, 0.1 Hz). For the GPI assay, the guinea pig ileum preparation was suspended with 0.5 g tension in a Krebs-Ringer solution and stimulated (30 V, 0.5 msec, 0.1 Hz). The contraction was recorded through an isometric transducer. These assays were accomplished in the presence of L-leucyl-L-leucine

(2 mM), together with bestatin (30 μM), thiorphan (0.3 μM), and captopril (10 μM). IC_{50} value is the concentration which inhibits the electrically stimulated muscle contraction by 50%.

Radioreceptor assay

The radioreceptor assays were performed according to the method of Pert and Snyder (12) using 1 nM [^{3}H]DAGO (47.8 Ci/mmol) or 1 nM [^{3}H]DADLE (30.0 Ci/mmol) as radioligands for μ- or δ-type opioid receptors, respectively in rat brain membranes. The radioreceptor assay for κ-receptors was performed using of 1 nM [^{3}H]EKC (24.5 Ci/mmol) and guinea pig cerebellum membranes as opioid receptor preparations. All assays were performed in the presence of the same protease inhibitors as used for the opioid activity assays. IC_{50} values are the concentrations of tested compounds inhibiting the binding of the labeled ligand by 50%.

Amino acid sequence analysis and peptide synthesis

The amino acid sequence of the purified peptide was analyzed by a 477A protein sequencer (Applied Biosystems Inc.). The peptide was synthesized by a Sam 2 peptide synthesizer (Biosearch Inc.).

Results

The enzymatic digests of wheat gluten inhibited the electrically stimulated contractions of the MVD (Fig. 1). However, these inhibiting activities were reversed in part by naloxone (opioid activity) and not reversed by naloxone (non opioid activity). The opioid activity in the pepsin-thermolysin digest or the pepsin-trypsin-chymotrypsin digest was higher than that in the pepsin digest. To isolate the opioid peptides, these digests were fractionated by reversed phase HPLC on an ODS column and the opioid activities of individual fractions were measured by the MVD assay. In the pepsin-thermolysin digest, four opioid active fractions (fractions I, II, III, IV) were recovered (Fig. 2-A), and in the pepsin-trypsin-chymotrypsin digest, one opioid active fraction (fraction V) was isolated (Fig. 2-B). However, the obvious opioid activity was not obtained from the pepsin digest. On the other hand, the non-opioid activity, which was not reversed by naloxone, was recovered from each of the three digests (Fig. 2-A, 2-B). This non-opioid substance was identified as adenosine by UV, NMR and mass spectra analyses. Authentic adenosine also inhibited the electrically stimulated contractions of the MVD and the effect was reversed by its antagonist, theophylline.

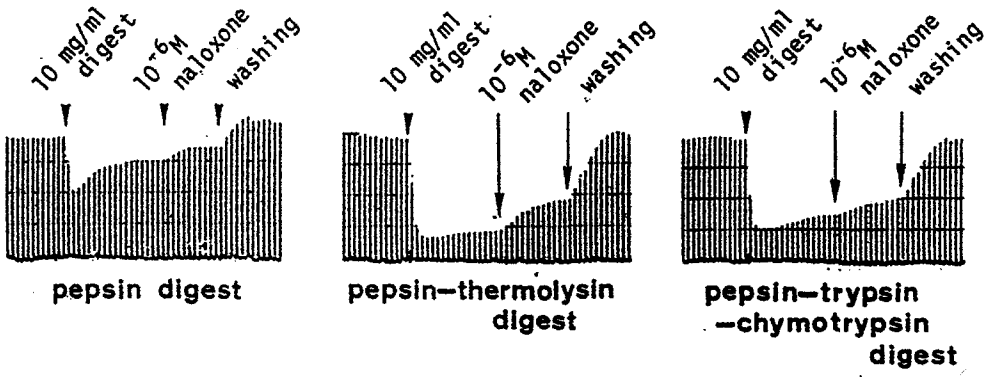

Fig. 1.: Effects of enzymatic digests of wheat gluten on the electrically stimulated contraction of MVD

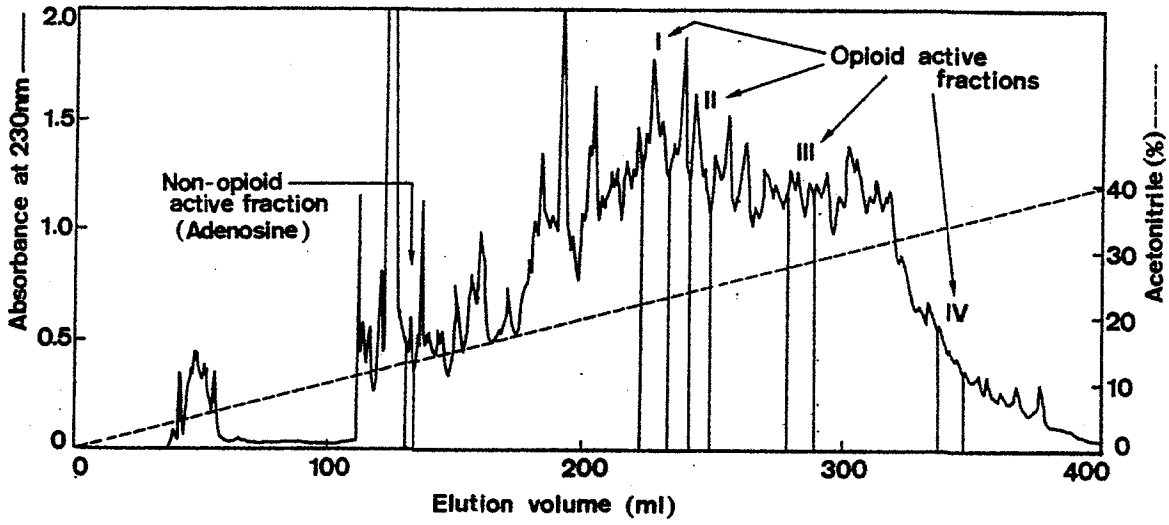

Fig. 2-A: Reversed phase HPLC yof the enzymatic digests on an ODS column, pepsin-thermolysin digest;

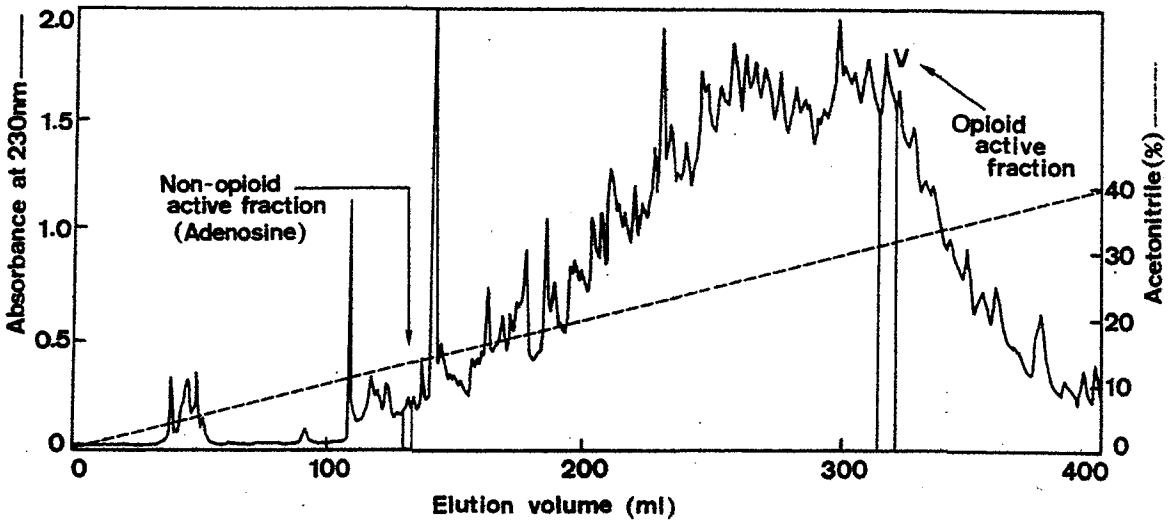

Fig. 2-B: Reversed please HPLC of the enzymatic digests on an ODS column, pepsin-trypsin-chymotrypsin digest

Five opioid-active fractions recovered from the ODS column were further purified by reversed phase HPLC on different columns and pure peptide fractions, fractions I-3, II-2, III-3, IV-5 and V-3 were obtained (Table 1). The structures of these peptides were analyzed with a protein sequencer and the sequences obtained were Gly-Tyr-Tyr-Pro-Thr (fraction I-3), Gly-Tyr-Tyr-Pro (fraction II-2), Tyr-Gly-Gly-Trp (fraction III-3), Tyr-Gly-Gly-Trp-Leu (fraction IV-5) and Tyr-Pro-Ile-Ser-Leu (fraction V-3), which were named gluten exorphins A5, A4, B4, B5 and C, respectively. From the sequence homology research, gluten exorphin A5/A4 were interspersed repeated sequences which were found 15 times in the primary structure of the high molecular weight glutenin (19) (Fig. 3). In addition, Arg-Tyr-Tyr-Pro, Ser-Tyr-Tyr-Pro, Trp-Tyr-Tyr-Pro, Gly-Tyr-Tyr-Leu and Gly-Tyr-Tyr-Ser, which were homologous to gluten exorphin A4, were also found in this primary structure. On the other hand, gluten exorphins B and C were not found in the primary structure reported of gliadin and glutenin (5, 13, 14, 18, 19).

	Number of opioid active fraction (%; acetonitrile)				
column / Opioid active fraction	Phenyl [1]	Cyano-propyl [1]	ODS [2]	ODS [1]	Phenethyl [2]
Pepsin-thermolysin digest					
I	I-1 (24.5)	I-2 (19)	I-3 (18)		
II	II-1 (33.5)	II-2 (27)			
III	III-1 (30)	III-2 (26)	III-3 (21)		
IV	IV-1 (36.5)	IV-2 (32.5)	IV-3 (28)	IV-4 (31)	IV-5 (29)
Pepsin-trypsin-chymotrypsin digest					
V	V-1 (33)	V-2 (27.5)	V-3 (24)		

Table 1:
Purification of opioid peptides derived from the pepsin-thermolysin and the pepsin-trypsin-chymotrypsin digests

This table shows a number of opioid active fractions which were obtained from fractions I, II, III IV (see also Fig. 2-A) and fraction V (see also Fig. 2-B), and the acetonitrile concentrations in which they were eluted in various reversed phase HPLC.
1) in the presence of 0.05 % TFA
2) in the presence of 10 mM potassium-sodium phosphate buffer (pH 7)

signal ← → mature

```
                  20                  40                  60
MAKRLVLFVAVVVALVALTVAEGEASEQLQCERELQELQERELKACQQVMDQQLRDISPE
                  80                 100                 120
CHPVVVSPVAGQYEQQIVVPKGGSFYPGETTPPQQLQQRIFWGIPALLKRYYPSVTSPQQ
                 140                 160                 180
VSYYPGQASPQRPGQGQQPGQGQQSGQGQQGYYPTSPQQPGQWQQPEQGQPGYYPTSPQQ
                 200                 220                 240
PGQLQQPAQGQQPGQGQQGRQPGQGQPGYYPTSSQLQPGQLQQPAQGQQGQQPGQGQQGQ
                 260                 280                 300
QLGQGQQGYYPTSLQQSGQGQPGYYPTSLQQLGQGQSGYYPTSPQQPGQGQQPGQLQQPA
                 320                 340                 360
QGQQPEQGQQGQQPGQGQQGQQPGQGQQPGQGQPGYYPTSPQQSGQGQPGYYPTSSQQPT
                 380                 400                 420
QSQQPGQGQQGQQVGQGQQAQQPGQGQQPGQGQPGYYPTSPLQSGQGQPGYYLTSPQQSG
                 440                 460                 480
QGQQPGQLQQSAQGQKGQQPGQGQQPGQGQQGQQPGQGQQGQQPGQGQPGYYPTSPQQSG
                 500                 520                 540
QGQQPGQWQQPGQGQPGYYPTSPLQPGQGQPGYDPTSPQQPGQGQQPGQLQQPAQGQQGQ
                 560                 580                 600
QLAQGQQGQQPAQVQQGQQPAQGQQGQQLGQGQQGQQPGQGQQPAQGQQGQQPGQGQQGQ
                 620                 640                 660
QPGQGQQPGQGQPWYYPTSPQESGQGQQPGQWQQPGQWQQPGQGQPGYYLTSPLQLGQGQ
                 680                 700                 720
QGYYPTSLQQPGQGQQPGQWQQSGQGQHGYYPTSPQLSGQGQRPGQWLQPGQGQQGYYPT
                 740                 760                 780
SPQQSGQGQQLGQWLQPGQGQQGYYPTSLQQTGQGQQSGQGQQGYYSSYHVSVEHQAASL
                 800
KVAKAQQLAAQLPAMCRLEGGDEALSASQ
```

Fig. 3:
The primary structure of high molecular weight glutenin reported by Sugiyama et al.(19). Gluten exorphins A5/A4 are boxed. Homologous sequences of gluten exorphin A4 are underlined

These gluten exorphins including their analogues were synthesized, and their opioid activities were evaluated by the GPI and the MVD assays and their receptor affinities by the radioreceptor assay. (Table 2). Gluten exorphins A had high affinities for δ-receptors. They showed the opioid activities on the MVD assay but not on the GPI assay; they had almost the same activities as α-casein exorphin 6 (Arg-Tyr-Leu-Gly-Tyr-Leu) on the MVD assay (10). The activities of Tyr-Tyr-Pro-Thr and Tyr-Tyr-Pro, which lack an N-terminal Gly of gluten exorphin A5/A4, were very weak. In the case of the analogues of gluten exorphin A4, Arg-Tyr-Tyr-Pro, Ser-Tyr-Tyr-Pro and Trp-Tyr-Tyr-Pro, these activities were lower than that of gluten exorphin A4. Especially, Trp-Tyr-Tyr-Pro had an opioid antagonist activity on the MVD assay. Its pA2 value was 3.3. Among the gluten exorphins A, B and C, the most potent opioid peptide was gluten exorphin B5; its IC_{50} values were 0.05 μM and 0.017 μM on the GPI and the MVD assays, respectively. The activity of gluten exorphin B4 was lower than that of gluten exorphin B5. This was especially remarkable in the MVD assay. The affinity of gluten exorphin B5 for κ-receptors was remarkably less than those for μ- and δ-receptor; its IC_{50} value was 30 μM. The opioid activity of gluten exorphin C was higher than those of gluten exorphins A; its IC_{50} values were 40 μM and 13.5 μM on the GPI and MVD assays, respectively.

Peptides	Receptor affinities IC_{50} values (μM)			Opioid activities IC_{50} values (μM)		
	[^{3}H]DAGO μ	[^{3}H]DADLE δ	μ/δ	GPI μ	MVD δ	μ/δ
Gly-Tyr-Tyr-Pro (Gluten exorphin A4)	>1000	3.8	—	>1000	70	—
Gly-Tyr-Tyr-Pro-Thr (Gluten exorphin A5)	700	1.5	467	1000	60	16.7
Gly-Tyr-Tyr-Pro-Thr-Ser	1010	5.0	202	>1000	72	—
Arg-Tyr-Tyr-Pro	—	—	—	>1000	190	—
Ser-Tyr-Tyr-Pro	>1000	5.6	—	>1000	200	—
Trp-Tyr-Tyr-Pro	700	100	7	—	a)	—
Tyr-Tyr-Pro	>1000	100	—	>1000	1000	—
Tyr-Tyr-Pro-Thr	—	—	—	—	800	—
Gly-Tyr-Tyr	>1000	500	—	>1000	1000	—
Tyr-Gly-Gly-Trp (Gluten exorphin B4)	0.17	0.18	0.94	1.5	3.4	0.44
Tyr-Gly-Gly-Trp-Leu (Gluten exorphin B5)	0.045	0.005	9	0.05	0.017	2.9
Tyr-Gly-Gly-Phe-Leu ([Leu]enkephalin)	0.037	0.003	12.3	0.04	0.004	10
Tyr-Pro-Ile-Ser-Leu (Gluten exorphin C)	—	—	—	40	13.5	2.96

Table 2:
Opioid activities and receptor affinities of gluten exorphins A, B, C and their analogues
a) This peptide displayed opioid antagonist activity on the MVD assay. (pA2 value = 3.3)

Discussion

We recognized opioid activity in a wheat gluten pepsin digest as well as other investigators did; however, we could not isolate the active peptide because of its very weak activity. Gluten exorphins A, B and C were isolated from the digests which were obtained by further hydrolysis of the pepsin digest with neutral proteases as thermolysin or trypsin and chymotrypsin. The opioid activities in the digests increased upon these treatments. This result suggest that, *in vivo*, the release of these peptides from a gastric pepsin digest might be caused by the actions of intestinal proteases and peptidases or enterobacterial proteases. It is suggested that the action of enterobacterial proteases might be necessary for the release of ß-casomorphin from casein (7). As for the origin of gluten exorphins A, B and C, gluten exorphin A sequences were recognized in wheat glutenin, but gluten exorphin B and C sequences were not recognized in wheat gliadin and glutenin of which the primary structures were reported. However, wheat gluten is a complex mixture of many different proteins. It is possible that gluten exorphins B and C are derived from a wheat protein of which the primary structure has not yet been determined.

These opioid peptides have characteristic structure-activity relationships. In gluten exorphins A, a N-terminal Gly are important for the expression of their activities. With this respect, their structure-activity relationships are quite different from that of endogenous opioid peptides in which an amino acid attached to the amino group of Tyr impaires the activity. Moreover, their structures are also unique in view of their Tyr-Tyr sequence. The structure of gluten exorphin B5 corresponds to [Trp^4, Leu^5] enkephalin. This peptide has been synthesized in the study of the structure-activity relationships of enkephalin derivatives (15). This peptide is similar to [Leu]enkephalin in that Leu^5 is important for the potency of δ-receptors (Table 2). It is the most potent among all the exogenous opioid peptides ever reported. In the structure of gluten exorphin C, the N-terminal Tyr residue is the only aromatic amino acid. Generally, endogenous and exogenous opioid peptides have one Tyr and further aromatic amino acids in their structures. In this respect, this is the most peculiar opioid peptide ever known.

The characteristic structure-activity relationships observed in this study are summarized as follows. Endogenous and exogenous opioid peptides can be classified into four groups according to the number of aromatic residues and the distance between them (Table 3). Gluten exorphins A, B and C are classfied in Tyr-aromatic, Tyr-X_1-X_2-aromatic and Tyr-X-aliphatic types, respectively. The structures of opioid peptides reported so far generally are of the Tyr-X_1-X_2-aromatic type such as the enkephalins, α-casein exorphins, ß-casorphin and α-lactorphins or of the Tyr-X-aromatic type such as ß-casomorphins, cytochrophins, hemorphins and serorphin. These structures have been considered to be essential for the opioid activities. In this respect, Tyr-aromatic and Tyr-X-aliphatic types such as gluten exorphins A and C provide a novel concept for structure-activity relationships of opioid peptides.

In the present study, we isolated five opioid peptides from the enzymatic digests of wheat gluten and established a new structural option for the expression of the opioid activity.

Peptides	Structures	Authors
Tyr-X_1-X_2-Aromatic		
[Met/Leu]enkephalin	Tyr-Gly-Gly-Phe-Met/Leu	Hughes (9)
α-casein exorphin	Arg-Tyr-Leu-Gly-Tyr-Leu-Glu	Loukas (10)
β-casorphin	Tyr-Pro-Ser-Phe-NH_2	Yoshikawa (21)
α-lactorphin	Tyr-Gly-Leu-Phe-NH_2	Yoshikawa (21)
gluten exorphin B4/B5	Tyr-Gly-Gly-Trp(-Leu)	present study
Tyr-X-Aromatic		
β-casomorphin (bovine)	Tyr-Pro-Phe-Pro-Gly-Pro-Ile	Brantl (1)
(human)	Tyr-Pro-Phe-Val-Glu-Pro	Yoshikawa (20)
cytochrophin	Tyr-Pro-Phe-Thr-Ile	Brantl (2)
hemorphin	Tyr-Pro-Trp-Thr-Gln	Brantl (3)
serorphin	Tyr-Gly-Phe-Gln-Asn-Ala	Yoshikawa (22)
Tyr-Aromatic		
gluten exorphin A4/A5	Gly-Tyr-Tyr-Pro(-Thr)	present study
Tyr-X-Aliphatic		
gluten exorphin C	Tyr-Pro-Ile-Ser-Leu	present study

Table 3:
The structures of endogenous and exogenous opioid peptides

Acknowledgement

We thank Dr. R. Sasaki at Kyoto University, H. Motoi and I. Kitamura at Nisshin Flour Milling Co., Ltd. for helpful discussions, and S. Hiramoto for NMR spectrum analysis.

References

1. Brantl, V.; Teschemacher, H.; Henschen, A.; Lottspeich, F. Novel opioid peptides derived from casein (ß-casomorphins). Hoppe-Seyler's Z. Physiol. Chem. 360: 1211-1216; 1979.
2. Brantl, V.; Gramsch, C.; Lottspeich, F.; Henschen, A.; Jaeger, K.H.; Herz, A. Novel opioid peptides derived from mitochondrial cytochrome b: cytochrophins. Eur. 1. Pharmacol. 111: 293-294; 1985.
3. Brantl, V.; Gramsch, C.; Lottspeich, F.; Mertz, R.; Jaeger, K. H.; Herz, A. Novel opioid peptides derived from hemoglobin: hemorphins. Eur. 1. Pharmacol. 125: 309-310; 1986.
4. Chiba, H.; Yoshikawa, M. Biologically functional peptides from food proteins: New opioid peptides from milk proteins. In: Feeny, R.E.; Whitaker, J.R., eds. Protein tailoring for food and medical uses. New York: Marcel Dekker, Inc. Press: 1986: 123-153.
5. Forde, I.; Forde, B.G.; Fry, R.P.; Kreis, M.; Shewry, P.R.; Miflin, B.J. Identification of barley and wheat cDNA clones related to the high-Mr polypeptides of wheat gluten. FEBS Lett. 162: 360-366; 1983.
6. Graf, L.; Horvath, K.; Walcz, E.; Berzetei, I.; Burnier, J. Effect of two synthetic α-gliadin peptides on lymphocytes in celiac disease: Identification of a novel class of opioid receptors. Neuropeptides 9: 113-122; 1987.
7. Hamel, U.; Kielwein, G.; Teschemacher, H. ß-Casomorphin immunoreactive materials in cows' milk incubated with various bacterial species. J. Dairy Res. 52: 139-148; 1985.
8. Huebner, F.R.; Lieberman, K.W.; Rubino, R.P.; Wall, J.S. Demonstration of high opioid-like activity in isolated peptides from wheat gluten hydrolysates. Peptides 5: 1139-1147; 1984.
9. Hughes, J.; Smith, T.W.; Kosterlits, H.W.; Fothergill, L.A.; Morgan, B.A.; Morris, R.H. Identification of two related pentapeptides from the brain with potent opiate agonist activity. Nature 258: 577-579; 1975.

10. Loukas, S.; Varoucha, D.; Zioudrou, C.; Streaty, R.A.; Klee, W.A., Opioid activities and structures of α—casein-derived exorphins. Biochemistry 22: 4567-4573; 1983.
11. Morley, J.E.; Levine, A.S.; Yamada, T.; Gebhard, R.L.; Prigge, W.F.; Shafer, R.B.; Goetz, F.C.; Silvis, S. E. Effect of exorphins on gastrointestinal function, hormonal release, and appetite. Gastroenterology 84: 1517-1523; 1983.
12. Pert, C.B.; Snyder, S.H. Properties of opiate-receptor binding in rat brain. Proc. Nat. Acad. Sci. USA 70: 2243-2247; 1973.
13. Rafalski, J.A.; Scheets, K.; Metzler, M.; Peterson, D.M.; Hedgcoth, C.; Soll, D.G. Developmentally regulated plant genes: the nucleotide sequence of a wheat gliadin genomic clone. EMBO J. 3: 1409-1415; 1984.
14. Rafalski, J.A. Structure of wheat gamma-gliadin genes. Gene 43: 221-229; 1986.
15. Schiller, P.W.; Yam, C.F.; Prosmanne, J. Synthesis, opioid receptor affinity, and conformational parameters of [4-Tryptophan]enkephalin analogues. J. Med. Chem. 21: 1110-1116; 1978.
16. Schusdziarra, V.; Henrichs, I.; Holland, A.; Klier, M.; Pfeiffer, E.F. Evidence for an effect of exorphins on plasma insulin and glucagon levels in dogs. Diabetes 30: 362-364; 1981.
17. Schusdziarra, V.; Schick, R.; Holland, A.; de la Fuente, A.; Specht, J.; Maier, V.; Brantl, V.; Pfeiffer, E.F. Effect of opiate-active substances on pancreatic polypeptide levels in dogs. Peptides 4: 205-210; 1983.
18. Shewry, P.R.; Autran, J.C.; Nimmo, C.C.; Lew, E.J.L.; Kasarda, D.D. N-terminal amino acid sequence homology of storage protein components from barley and a diploid wheat.Nature 286: 520-522; 1980.
19. Sugiyama, T.; Rafalski, A.; Peterson, D.; Soll, D. A wheat HMW glutenin subunit gene reveals a highly repeated structure. Nucleic Acids Res. 13: 8729-8737; 1985.
20. Yoshikawa, M.; Yoshimura, T.; Chiba, H. Opioid peptides from human ß-casein. Agric. Biol. Chem. 48: 3185-3187; 1984.
21. Yoshikawa, M.; Tani, F.; Yoshimura, T.; Chiba, H. Opioid peptides from milk proteins Agric. Biol. Chem. 50: 2419-2421; 1986.
22. Yoshikawa, M.; Tani, F.; Shiota, A.; Chiba, H. Serorphin, an opioid peptide derived from bovine serum albumin. In: Brantl, V., Teschemacher, H., eds. ß-Casomorphins and Related Peptides. Weinheim, VCH Verlag III, this volumne.
23. Zioudrou, C.; Streaty, R.A.; Klee, W.A. Opioid peptides derived from food proteins (The exorphins). J. Biol. Chem. 254: 2446-2449; 1979.