Volume 316, number 1, 17–19 FEBS 11997 January 1993

Gluten exorphin C

A novel opioid peptide derived from wheat gluten

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Received 5 November 1992; revised version received 30 November 1992

A novel opioid peptide, Tyr-Pro-Ile-Ser-Leu, was isolated from the pepsin-trypsin-chymotrypsin digest of wheat gluten. Its IC_{50} values were 40 μM and 13.5 μM in the GPI and MVD assays, respectively. This peptide was named gluten exorphin C. Gluten exorphin C had a structure quite different from any of the endogenous and exogenous opioid peptides ever reported in that the N-terminal Tyr was the only aromatic amino acid. The analogs containing Tyr-Pro-X-Ser-Leu were synthesized to study its structure–activity relationship. Peptides in which X was an aromatic amino acid or an aliphatic hydrophobic amino acid had opioid activity.

Opioid peptide; Wheat; Gluten; Exorphin

1. INTRODUCTION

The presence of the opioid peptides has been recognized in the pepsin digest of wheat gluten [1,2]. However, there was no report about their structure and character. Recently, we observed that the opioid activity in the digest obtained by further hydrolyses of the pepsin digest with microbial protease thermolysin was more potent than that in the pepsin digest and four novel opioid peptides, Gly-Tyr-Tyr-Pro-Thr, Gly-Tyr-Tyr-Pro, Tyr-Gly-Gly-Trp-Leu and Tyr-Gly-Gly-Trp, were isolated from the pepsin–thermolysin digest [3]. They were named gluten exorphins A5, A4, B5 and B4, respectively. On the other hand, we also found high-opioid activity in the digest treated with only gastrointestinal proteases, pepsin, trypsin and chymotrypsin [3]. In this study, we isolated a new opioid peptide, which had a peculiar structure–activity relationship, from the pepsin–trypsin–chymotrypsin digest of wheat gluten.

2. MATERIALS AND METHODS

2.1. *Chemicals and reagents*

Wheat gluten was obtained from Goodman Fielder Mills Ltd. Pepsin, trypsin and chymotrypsin were from Sigma Chemical Co. Naloxone was from U.S.P.C., Inc. [^{3}H]DAGO and [^{3}H]DADLE were from Amersham. Other reagents used were of reagent grade or better.

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Abbreviations: GPI, guinea pig ileum; MVD, mouse vas deferens; DAGO, [DAla^2,MePhe^4,Glyol^5]enkephalin; DADLE, [DAla^2,DLeu^5] enkephalin; ODS, octadecyl silica; HPLC, high-performance liquid chromatography; TFA, trifluoroacetic acid.

2.2. *Enzymatic digestion of wheat gluten*

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

2.3. *Purification of peptides*

Separation of peptides in the digest was accomplished by reversed-phase HPLC on an ODS column (Cosmosil 5C_{18}-AR, 20 × 250 mm, Nacalai Tesque Inc.). A 100 mg digest was applied to the column and eluted with a linear gradient between 0 to 40% acetonitrile containing 0.05% TFA at 10 ml/min. The eluate was monitored at 230 nm. Individual fractions were dried with a centrifugal concentrator and their opioid activities were measured with the MVD assay. The opioid active fractions were purified on an ODS column (Cosmosil 5 C_{18}-AR, 4.6 × 150 mm), a phenyl silica column (Cosmosil 5Ph, 4.6 × 250 mm) and cyanopropyl silica column (Cosmosil 5CN-R, 4.6 × 250 mm) from Nacalai Tesque Inc. The columns were developed by a linear gradient between 0 to 50% acetonitrile containing 0.05% TFA or 10 mM potassium–sodium phosphate buffer (pH 7) at 1 ml/min. The eluate was monitored at 215 nm to 235 nm. As a first step, the active fractions were purified on the same ODS column.

2.4. *Amino acid sequence analyses and peptide synthesis*

The amino acid sequence of the purified peptide was analyzed by a 477A protein sequencer (Applied Biosystems Inc.). Peptides were synthesized by Sam 2 peptide synthesizer (Biosearch Inc.) according to the *t*-butyloxycarbonyl strategy. Peptides were deprotected by the anisol/hydrogen fluoride method and purified by reversed-phase HPLC on an ODS column. The structures of synthesized peptides were confirmed by a protein sequencer.

2.5. *Opioid activity assays and radioreceptor assays*

Opioid activity assays and radioreceptor assays were performed as previously described [3].

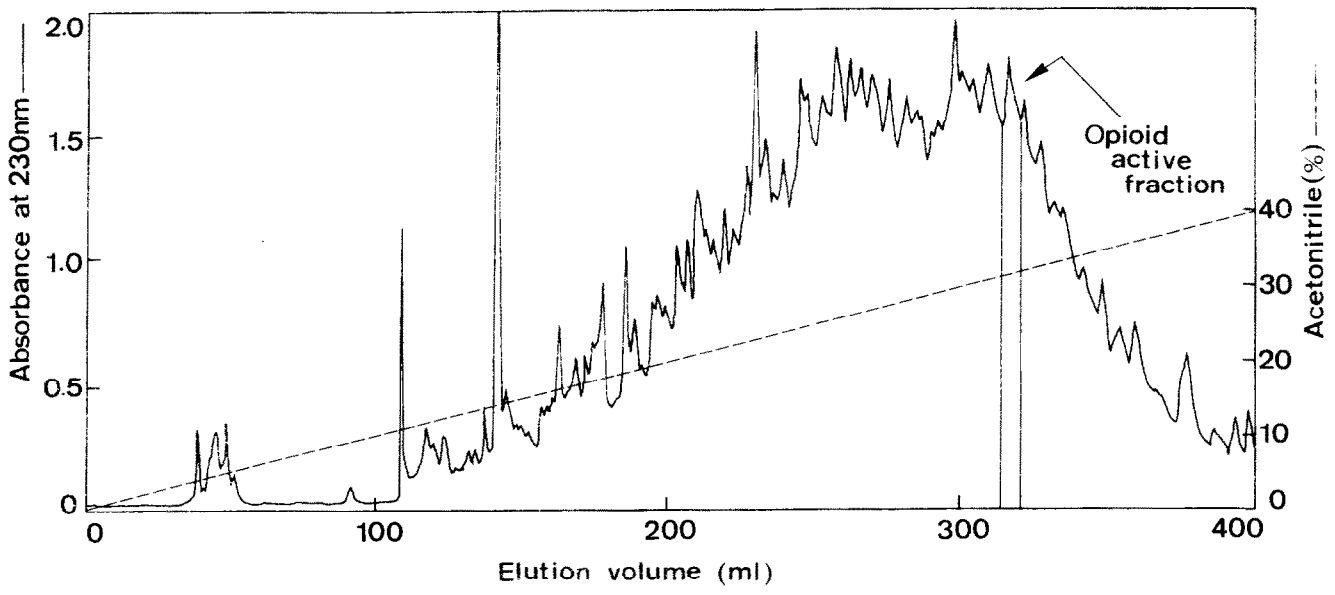

Fig. 1. Reversed-phase HPLC of the pepsin–trypsin–chymotrypsin digest on an ODS column.

3. RESULTS AND DISCUSSION

In the previous paper, we reported the presence of opioid activity in the pepsin–trypsin–chymotrypsin digest of wheat gluten using the MVD assay [3]. This digest was fractionated by reversed-phase HPLC on an ODS column and opioid activities of the individual fractions were measured using the MVD assay. The opioid activity was eluted at about 31 to 32% acetonitrile (Fig. 1, Table I). This opioid active fraction from the ODS column was purified by reversed-phase HPLC on the other columns (Table I). The purified peptide was analysed with a protein sequencer and the sequence obtained was Tyr-Pro-Ile-Ser-Leu. This peptide was named gluten exorphin C. The yield of this peptide against wheat gluten was 4×10^{-4}%. However, the sequence which corresponded to gluten exorphin C was not found in the primary structure of gliadin or glutenin reported until now. This peptide might be derived from a wheat protein of which the primary structure has not yet been determined.

Table I

Purification of the opioid peptide derived from the pepsin–trypsin–chymotrypsin digest of wheat gluten. This table shows the acetonitrile concentrations in which opioid active fractions were eluted in various reversed-phase HPLC.

Column	ODS[a]	Phenyl[a]	Cyano-propyl[a]	ODS[b]
Column size (mm)	20 × 250	4.6 × 250	4.6 × 250	4.6 × 150
Elution condition (% acetonitrile)	31.5	33.0	27.5	24.0

[a] In the presence of 0.05% TFA
[b] In the presence of 10 mM potassium–sodium phosphate buffer (pH 7).

Gluten exorphin C was isolated from the digest which is hydrolyzed with only gastro-intestinal proteases, pepsin, trypsin and chymotrypsin. This means that this peptide is certainly released in digestive organs after the ingestion of wheat gluten. It has been reported that the oral administration of the peptic digest of wheat gluten influenced the regulation of gastro-intestinal motility and hormone release, and these effects were reversed by naloxone [4–6]. In this respect, it might be possible that gluten exorphin C is responsible for such a regulation of physiological functions.

We synthesized gluten exorphin C and evaluated its opioid activities by the GPI and MVD assays and its receptor affinities by radioreceptor assays. Its IC_{50} values were 40 μM and 13.5 μM in the GPI and the MVD assays, respectively (Table II). Together with the radioreceptor assay results, this peptide was a rather δ-selective ligand. All of the endogenous and exogenous opioid peptides ever reported have an N-terminal Tyr and a second aromatic amino acid residue at positions 2 or 3. Especially, in enkephalins (Tyr-Gly-Gly-Phe-Met/ Leu), it is reported that Phe at position 4 was important for δ-receptor affinity [7]. In addition, exogenous opioid peptides containing the Tyr-Pro sequence, δ-casomorphin 7 (Tyr-Pro-Phe-Pro-Gly-Pro-Ile), hemorphin 4 (Tyr-Pro-Trp-Thr) and cytochrophin 4 (Tyr-Pro-Phe- Thr) have a second aromatic amino acid residue such as Phe or Trp at position 3 [8–10]. In this respect, gluten exorphin C is a characteristic opioid peptide in that the N-terminal Tyr is the only aromatic amino acid residue in the structure.

Therefore, we synthesized the analogs of gluten exorphin C of which the structure was displayed as Tyr-Pro-

Table II

Opioid activities and receptor affinities of gluten exorphin C and its analogs

Peptides	Opioid activities (IC_{50})			Receptor affinities (IC_{50})		
	GPI	MVD	μ/δ	[^{3}H]DAGO	[^{3}H]DADLE	μ/δ
	μ (μM)	δ (μM)		μ (μM)	δ (μM)	
Tyr-Pro-Ile-Ser-Leu (gluten exorphin C)	40	13.5	3.0	110	30	3.7
Tyr-Pro-Val-Ser-Leu	200	200	1.0	350	150	2.3
Tyr-Pro-Leu-Ser-Leu	200	200	1.0	540	110	4.9
Tyr-Pro-Lys-Ser-Leu	>1000	>1000	–	>1000	>1000	–
Tyr-Pro-Ala-Ser-Leu	>1000	>1000	–	940	500	1.9
Tyr-Pro-Thr-Ser-Leu	>1000	>1000	–	>1000	400	–
Tyr-Pro-Nva-Ser-Leu	33	14.0	2.4	200	74	2.7
Tyr-Pro-Nle-Ser-Leu	30	15.0	2.0	96	70	1.4
Tyr-Pro-Phe-Ser-Leu	30	70	0.4	64	38	1.7
Tyr-Pro-Trp-Ser-Leu	20	70	0.3	13	8	1.6

X-Ser-Leu to study its structure–activity relationship and evaluate their opioid activities and receptor affinities (Table II). The analogs in which X were aliphatic hydrophobic amino acids (Val, Leu) were less potent than gluten exorphin C. In the case when X was a straight chain aliphatic hydrophobic amino acid (Nva or Nle), their potencies were almost the same as gluten exorphin C. On the other hand, the analogs in which X was a basic or hydrophilic amino acid (Lys, Ala, Thr) had almost no activity. β-Casomorphin 7- and hemorphin 4-like analogs in which X was replaced by an aromatic amino acid (Phe or Trp) showed almost the same activity to gluten exorphin C in the GPI assay.

These results can be summarized by stating that the Tyr-Pro-X-Ser-Leu analogs in which X was represented by an aliphatic hydrophobic amino acid or an aromatic amino acid had opioid activity and that the variation of the hydrophobic amino acid at position 3 greatly affected their opioid activity. In this respect, it was suggested that the hydrophobicity of Ile^3 was important for the expression of the opioid activity of gluten exorphin C. From the present study of the isolation and of the structure–activity relationship of gluten exorphin C, it is concluded that the second aromatic amino acid at position 3 is not essential for the opioid activity of peptides having the Tyr-Pro sequence.

Acknowledgements: We thank Prof. R. Sasaki at Kyoto University, H. Motoi and I. Kitamura at Nisshin Flour Milling Co., Ltd. for their helpful discussions.

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