Description

PHARMACEUTICAL COMPOSITION CONTAINING DIPEPTIDE

Technical Field

[0001]

The present invention relates to a pharmaceutical or a food product composition containing a dipeptide.

Background Art

[0002]

Super-aging society increases the importance of an effort of prevention and treatment of lifestyle-related diseases such as metabolic diseases including diabetes and obesity and circulatory diseases including arteriosclerosis, with which chronic inflammation is related. In reality, inhibitory actions on IL-1β signal and NF-κB have been developed to counteract diabetes. In the chronic inflammatory diseases represented by rheumatoid arthritis, abnormally large amounts of inflammatory cytokines such as TNF-α, IL-1, and IL-6 are produced and secreted and inflammatory cytokine inhibiting drugs therefor are useful as good anti-inflammatory agents. For example, antibody drugs such as infliximab have been used as TNF-α inhibiting drugs to treat rheumatoid arthritis (Non Patent Literature 1). An antibody drug, tocilizumab, has been used as an IL-6 inhibiting drug (Non Patent Literature 2). Protein

preparations and antibody drugs such as Anakinra, Rilonacept, and Canakinumab have been developed as IL-1 inhibiting drugs (Non Patent Literature 3).

Citation List

Non Patent Literature

[0003]

Non Patent Literature 1: The guideline about the usage of TNF inhibitors on rheumatoid arthritis (RA) (Japan College of Rheumatology)

Non Patent Literature 2: Journal of the Pharmaceutical Society of Japan 129(6) 667-674(2009)

Non Patent Literature 3: The Journal of the Japanese Society of Internal Medicine, Vol. 100, No. 10, p. 2985-2990 (October 10, 2011)

Summary of Invention

Technical Problem

[0004]

Under the circumstances, the present inventors found that a liver hydrolysate has a good action of suppressing IL-1 production and are useful as an agent for suppressing cytokine production and an anti-inflammatory agent and filed earlier a patent application (PCT/JP2018/006351).

A liver hydrolysate is considered to contain abundant amino acids and peptides, however, the actual active component has not been revealed.

Thus, an object of the present invention is to provide a novel anti-inflammatory agent based on the action of suppressing cytokine production.

Solution to Problem

[0005]

The present inventors hence studied to develop a novel anti-inflammatory agent, investigated for indigestible peptides which enter the blood by administering a liver hydrolysate to an animal and evaluated pharmacological effects by fractionating peptides of a liver hydrolysate using various columns and, as a result, found that dipeptides derived from specific D-amino acids, not from an L-amino acid, and also that the dipeptides have an action of suppressing production of cytokines such as IL-1β and IL-6 whereby the present invention was accomplished.

[0006]

More specifically, the present invention provides the following [1] to [15].

[0007]

[1] An anti-inflammatory agent comprising as an active component a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-

(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[2] The anti-inflammatory agent according to [1], wherein the active component is selected from the group consisting of (D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[3] An agent for suppressing inflammatory cytokine production comprising as an active component a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[4] The inflammatory cytokine production-suppressing agent according to [3], wherein the active component is selected from the group consisting of (D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[5] An agent for suppressing IL-1 production or an agent for suppressing IL-6 production comprising as an active component a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-

(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[6] The agent for suppressing IL-1 production or the agent for suppressing IL-6 production according to [5], wherein the active component is selected from the group consisting of (D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[7] A food product composition for alleviating inflammation, comprising as an active component a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[8] The food product composition according to [7], wherein the active component is selected from the group consisting of (D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-

(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[9] A food product composition for suppressing inflammatory cytokine production, comprising as an active component a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[10] The food product composition according to [9], wherein the active component is selected from the group consisting of (D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[11] A food product composition for suppressing IL-1 production or a food product composition for suppressing IL-6 production, comprising as an active component a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val,

(D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[12] The food product composition according to [11], wherein the active component is selected from the group consisting of (D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[13] Use of a dipeptide for producing an anti-inflammatory agent selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[14] A dipeptide for use in treatment of an inflammatory disease, wherein the dipeptide is selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[15] A method for treating an inflammatory disease, comprising administering an effective amount of a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

Advantageous Effect of Invention

[0008]

The dipeptides of the present invention are indigestible as they are derived from D-amino acids, stay in blood for an extended period of time and have an IL-1 production-suppressing action and an IL-6 production-suppressing action thereby being useful as pharmaceutical and food product compositions for suppression of cytokine production and anti-inflammation.

Brief Description of Drawings

[0009]

[Figure 1] Figure 1 shows an action of suppressing IL-6 production in a hydrophobic peptide fraction.

[Figure 2] Figure 2 shows an action of suppressing IL-6 production in a hydrophobic pyroglutamyl peptide fraction.

[Figure 3] Figure 3 shows an action of suppressing IL-6 production in a hydrophilic peptide fraction.

[Figure 4] Figure 4 shows an action of suppressing IL-6 production in a hydrophilic pyroglutamyl peptide fraction.

[Figure 5] Figure 5 shows an action of suppressing IL-1β production in a hydrophobic peptide fraction.

[Figure 6] Figure 6 shows an action of suppressing IL-1β production in a hydrophobic pyroglutamyl peptide fraction.

Description of Embodiments

[0010]

The dipeptide which is an active component of the anti-inflammatory agent of the present invention is a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe, among which a dipeptide selected from the group consisting of D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-

(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe is preferable from the viewpoint of ability to enter the blood when orally administered, action sustainability and anti-inflammatory action, and (D)Asp-(D)Leu and (D)Asp-(L)Leu are further preferable.

(D) as represented herein means that an amino acid is the D-amino acid. (L) as represented means that an amino acid is the L-amino acid.

Further, among the α-amino acid and the β-amino acid of the above (D)- or (L)-dipeptides, the β-amino acid is more preferable.

[0011]

The dipeptide of the present invention can be produced using a (D)-amino acid as a starting material by a typical liquid-phase peptide synthesis method or solid-phase peptide synthesis method. For example, an amino acid in which the functional groups other than the α-amino group are protected and an amino acid in which the functional groups other than the carboxyl group are protected or an amino acid in which the carboxyl group is activated and in which the functional groups other than the carboxyl group are protected are condensed, and subsequently the protecting groups are deprotected to produce the dipeptide.

Examples of the protecting group for the amino group of an amino acid herein include a benzyloxycarbonyl group, a tert-butoxycarbonyl group, a

fluorenylmethoxycarbonyl group, and the like. Examples of the protecting group for the carboxyl group include a tert-butyl group, a benzyl group, and the like. The condensation reaction can employ a method of using N,N'-dicyclohexylcarbodiimide, dicyclohexylurea, or other condensation agents, an active ester method by use of, for example, nitrophenol and N-hydroxysuccinimide, a mixed acid anhydride method, or the like.

The protecting groups are removed after completing the condensation reaction and, in the case of the solid-phase method, the link between the C-terminal end of the peptide and a resin is further cleaved. Further, the peptide of the present invention is purified in accordance with a typical method.

[0012]

The dipeptide of the present invention can be an acid addition salt or a base salt. Examples of the acid addition salt include a salt of an inorganic acid such as hydrochloric acid, sulfuric acid, nitric acid, phosphoric acid, hydrobromic acid, perchloric acid, and the like and a salt of an organic acid such as citric acid, succinic acid, maleic acid, fumaric acid, malic acid, tartaric acid, p-toluenesulfonic acid, benzenesulfonic acid, methanesulfonic acid, trifluoroacetic acid, and the like. Examples of the base salt include a salt of an alkali metal such as sodium, potassium, lithium, and the like and a salt of an alkaline earth metal such as calcium, magnesium, and the like.

[0013]

The peptide of the present invention can be a solvate. Examples of the solvate include a solvate of water (in the case of a hydrate), methanol, ethanol, isopropanol, and the like.

[0014]

The peptide of the present invention is indigestible with good ability to enter the blood after orally administered and good action sustainability and has an action of strongly suppressing production of IL-1β and IL-6 which are one of inflammatory cytokines and an action of suppressing mRNA expression of IL-1β and IL-6. Thus, the dipeptide suppresses production of inflammatory cytokines represented by IL-1 and IL-6, and is useful as an agent for treating an inflammatory disease to which inflammatory cytokines are related and useful as a food and drink composition for alleviating inflammation. Here, examples of the disease to which inflammatory cytokines such as IL-1 and IL-6 are related include rheumatoid arthritis, osteoarthrosis, an inflammatory enteric disease, sepsis, acute or chronic myeloid leukemia, osteoporosis, a lifestyle-related disease, and the like.

[0015]

The pharmaceutical composition of the present invention can be administered by oral administration, transdermal administration, enteral administration, intravenous administration, or the like, and oral

administration is more preferable. Examples of the formulation for oral administration include a liquid preparation, a tablet, a powder, a fine granule, a granule, a capsule, and the like, with a liquid preparation and a tablet being preferable, and a liquid preparation being more preferable.

[0016]

These oral administration formulations can be prepared by using an excipient such as lactose, mannitol, cornstarch, or crystalline cellulose, a binder such as a cellulose derivative, gum arabic, or gelatin, a disintegrating agent such as calcium carboxymethylcellulose, a lubricant such as talc or magnesium stearate, a solubilizing agent such as a nonionic surfactant, a flavoring agent, a sweetener, a stabilizer, a pH adjuster, water, ethanol, propylene glycol, glycerine, or the like. Additionally, a coating agent such as hydroxymethyl cellulose phthalate, hydroxypropyl methylcellulose acetate succinate, cellulose acetate phthalate, or a methacrylate copolymer may also be used.

[0017]

Further, the pharmaceutical composition of the present invention can also contain other active components. Examples of the other active components include vitamin B_1 compounds such as thiamine, thiamine nitrate, thiamine chloride hydrochloride, fursultiamine, bisbentiamine, benfotiamine, thiamine disulfide,

dicethiamine, thiamine propyl disulfide, and a derivative thereof, vitamin B_2 compounds such as riboflavin and a derivative thereof and a salt thereof, vitamin B_3 compounds such as niacin, nicotinic acid, nicotinic acid amide, and a derivative thereof and a salt thereof, vitamin B_5 compounds such as panthenol, pantothenic acid, and a derivative thereof and a salt thereof, vitamin B_6 compounds such as pyridoxine and a derivative thereof and a salt thereof, vitamin B_{12} compounds such as cyanocobalamin and a derivative thereof and a salt thereof, other vitamins such as vitamin A, vitamin C, vitamin E, vitamin K, and vitamin P, diisopropylamine dichloroacetate, taurine, chondroitin sulfate, royal jelly, caffeine, turmeric, milk thistle, Japanese dandelion, dandelion, burdock, garlic, chrysanthemum, yarrow, common gardenia, sesame, notoginseng, asparagus, onion, chicory, common sage, artichoke, Chinese desert-thorn, plants of the family Fabaceae or the family Iridaceael, miyamauzura (*Goodyera schlechtendaliana*), Erva-de-passarinho, Sete-Sangria, Japanese Mallotus, black tea, resveratrol, catechin compounds, berberine, rosemary, bean extract, metformin, and the like.

[0018]

Further, the composition of the present invention can also be used as, in addition to a pharmaceutical product, a quasi-drug, a specific health food and a functional food such as a sports drink, a drink for rehabilitation, and a pet food.

[0019]

A content of the dipeptide in the pharmaceutical composition or the food product composition of the present invention varies depending on the dosage form and is typically preferably from 0.001 to 10 mass% and more preferably from 0.001 to 5 mass%. Additionally, a daily dose of the dipeptide in the pharmaceutical composition or the food product composition of the present invention is preferably from 10 mg to 1,000 mg, more preferably from 20 mg to 800 mg, and further preferably from 50 mg to 800 mg.

Example

[0020]

Subsequently, the present invention is described in detail in reference to examples but the present invention is not limited thereto.

[0021]

Example 1 (Fractionation of a liver hydrolysate)

(1) Fractionation of peptide and pyroglutamyl peptide

A strong cation exchange resin (AG50) was filled in an Econo Column (2.5 × 20 cm) and the resin was equilibrated with 10 mM HCl. In 20 mL of 10 mM HCl, 1 g of a liver hydrolysate (Sample A) was dissolved. The solution was added onto the resin and 20 mL of a flow-through fraction was collected (flow-through fraction 1). Subsequently, 20 mL of 10 mM HCl was added onto the resin and 20 mL of a flow-through fraction was collected. This

operation was repeated 19 times (flow-through fractions 2 to 20). The absorbance (230 nm) of the flow-through fractions 1 to 20 was measured to confirm the elution of the peptides.

Subsequently, 20 mL of a 50% ammonia solution was added onto the resin and 20 mL of an adsorbed fraction was collected. This operation was repeated 20 times (adsorbed fractions 1 to 20). The absorbance (230 nm) of the adsorbed fractions 1 to 20 was measured to confirm the elution of the peptides.

The flow-through fractions (pyroglutamyl peptide fractions) and the adsorbed fractions (peptide fractions) were concentrated under reduced pressure using an evaporator.

[0022]

(2) Hydrophilic and hydrophobic fractionation

A solid-phase extraction column (Sep-Pak) was equilibrated with 10 mM HCl. The pyroglutamyl peptide fraction was allowed to pass through the column to elute and collect a flow-through fraction (hydrophilic pyroglutamyl peptide fraction). Subsequently, a 60% acetonitrile solution containing 10 mM HCl was allowed to pass through the column to elute and collect an adsorbed fraction (hydrophobic pyroglutamyl peptide fraction).

The peptide fraction was also subjected to these operations.

The obtained 4 fractions were lyophilized.

[0023]

Example 2

(1) Quantitative experiment of indigestible peptides in the liver hydrolysate

1) In 1 mL of 50 mM Tris-HCl, 2.5 mg of a liver hydrolysate was dissolved.

2) Pancreatin (0.1 mg), leucine aminopeptidase (2.45 unit) and carboxypeptidase (7.7 unit) were added to 1) to cause an enzyme reaction (37°C, 24h).

3) The enzymes were removed by ultrafiltration (10K).

4) 3) was allowed to pass through a strong cation exchange resin (AG50) filled in a spin column to collect a flow-through fraction (pyroglutamyl peptide fraction).

5) By size exclusion HPLC (SEC Fr.35-44), 200 μL of 3) (peptide fraction) and 4) (pyroglutamyl peptide fraction) were fractionated and separated.

6) For the peptide fraction, SEC Fr.35-44 were solidified to dryness and derivatized with AccQ. For the pyroglutamyl peptide fraction, SEC Fr.35-44 were directly used.

7) The structure of indigestible peptide was determined by LC-MS/MS analysis.

Column: Inertsil ODS-3

Eluent: 0.1% formic acid and 80% acetonitrile containing 0.1% formic acid

Analysis method: for the peptide fraction, AccQ fragment (m/z = 171.1) was specifically detected (Precursor ion scan analysis), subsequently the structure

was estimated by MS/MS analysis to synthesize a standard whereby identification and quantification are carried out by MRM analysis.

For the pyroglutamyl peptide fraction, peaks are detected by total scan ion analysis, subsequently the structure was estimated by MS/MS analysis to synthesize a standard whereby identification and quantification are carried out by MRM analysis.

[0024]

(2) Experiment on ability of peptide to enter the blood when a liver hydrolysate was administered to a rat

1) A single dose of an aqueous solution of a liver hydrolysate was administered to a Wister rat (6-week old, male) (10 g/60 kg).

2) Blood was collected from the abdominal vena cava under isoflurane anesthesia 30 min and 60 min after the administration, to thereby obtain plasma. Further, the digestive tract (duodenum to ileum) is removed and luminal contents are dissolved out with 10 mL of saline. Three times the amount of ethanol was added to the plasma and the luminal contents. (preserved at -20°C until analyzed).

3) The ethanol supernatant of plasma and luminal contents was solidified to dryness and derivatized with AccQ.

4) Identification and quantification of the indigestible peptides identified in (1) were carried out by MRM analysis.

Dipeptide concentrations in blood are shown in Table 1.

[0025]

[Table 1]

(pmol mL)

(min)	Asp-Leu (D α)	Asp-Val (D α)	Asp-Ile (D α)	Asp-Phe (D α)
0	2.03±0.94	ND	2.03±0.94	0.09±0.16
60	16.43±2.09**	8.84±0.85**	16.43±2.10**	2.08±0.09**
(min)	Asp-Leu (D β)	Asp-Val (D β)	Asp-Ile (D β)	Asp-Phe (D β)
0	3.41±0.50	ND	3.56±1.28	1.54±0.62
60	28.54±2.83**	16.31±3.27*	26.37±2.66**	17.67±3.25**

Mean (n = 3) ± SD *p<0.05, **p<0.01

Asp-Leu (Dα), Asp-Val (Dα): As Asp-Ile and Asp-Leu were not separated by LC, both are Asp-Ile + Asp-Leu (Dα).

[0026]

As a result, it was revealed that (D)Asp-(D)Val, (D)Asp-(D)Phe, (D)Asp-(D)Ile, (D)Asp-(D)Leu, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe had good ability to enter the blood when orally administered and sustained in blood for an extended period of time.

[0027]

Example 3 (Synthesis of dipeptide)

1) Reagents were added to a round-bottom flask in the following order to cause a reaction while stirring (4°C, overnight).

(i) H-Leu-OtBu·HCl

(ii) DMF

(iii) TEA

(iv) Boc-Asp(OtBu)-OH (Lα amino acid)

(v) HOBt

(vi) EDL·HCl

In the case of other isomers, the following protected amino acids are used.

Boc-D-Asp(OtBu)-OH (Dα amino acid)

Boc-Asp-OtBu (Lβ amino acid)

Boc-D-Asp-OtBu (Dβ amino acid)

2) DMF is removed using an evaporator.

3) The resultant is dissolved in ethyl acetate and moved to a separatory funnel.

4) Thereto, 5 % sodium hydrogen carbonate is added, stirred and the aqueous layer is removed. (× 2)

5) Thereto, 10 % citric acid is added, stirred and the aqueous layer is removed. (× 2)

6) Thereto, saturated saline is added, stirred and the aqueous layer is removed.

7) The ethyl acetate layer is collected and sodium hydrogen sulfate is added to dehydrate.

8) The ethyl acetate layer is collected by filtration and concentrated using an evaporator.

9) Petroleum ether is added thereto and precipitation caused is dried. (When not caused, proceed to 10))

10) 4M HCl/dioxane is added to the dried product and reacted wile stirring. (4°C, 24 to 48h)

11) 4M HCl/dioxane is removed using an evaporator.

12) Diethyl ether is added thereto, the obtained precipitation is ultrasonically crushed and washed, and the ether supernatant is removed by decantation. (× 3)
13) Diethyl ether is added thereto and allowed to stand (4°C, overnight).
14) Diethyl ether is added thereto, the obtained precipitation is ultrasonically crushed and washed, and the ether supernatant is removed by decantation. (× 3)
15) The precipitate is dried.

[0028]

Example 4

RAW 264.7 cells were seeded such that the number of cells was adjusted to be 1.5×10^6 cells/dish and cultured overnight. A sample before fractionation and a filtrate fraction were both diluted 20-fold with EMEM medium, respectively (final concentration about 0.6 mg/mL). For control, PBS in place of the sample was diluted 20-fold with EMEM medium. Each of the above samples diluted with the medium (control, before fractionation, filtrate fraction) was added to each dish group. After 24-hr culture, lipopolysaccharide (LPS) was added so as to be the final concentration of 1.0 μg/mL. The dish groups were allowed to stand for 3 hours and RNA was collected to carry out cDNA synthesis by reverse transcription reaction. Subsequently, EEF1A1 and IL-6 were measured by RT-RCR.

[0029]

The results are shown in Figure 1 to Figure 4.

In the LPS-stimulated RAW cells, mRNA expression levels of inflammation-related genes were compared between the dipeptide groups and the Control group to which PBS was added in place of the dipeptide, and IL-6 was found to have been significantly reduced in the hydrophobic peptide fraction and the hydrophobic pyroglutamyl peptide fraction, thereby suggesting an effect of the dipeptides on suppression of inflammation.

[0030]

Example 5

RAW 264.7 cells were seeded such that the number of cells was adjusted to be 1.5×10^6 cells/dish and cultured overnight. A sample before fractionation and a filtrate fraction were both diluted 20-fold with EMEM medium, respectively (final concentration about 0.6 mg/mL). For control, PBS in place of the sample was diluted 20-fold with EMEM medium. Each of the above samples diluted with the medium (control, before fractionation, filtrate fraction) was added to each dish group. After 24-hr culture, lipopolysaccharide (LPS) was added so as to be the final concentration of 1.0 μg/mL. The dish groups were allowed to stand for 3 hours and RNA was collected to carry out cDNA synthesis by reverse transcription reaction. Subsequently, EEF1A1 and IL-1β were measured by RT-RCR.

[0031]

The results are shown in Figure 5 and Figure 6.

In the LPS-stimulated RAW cells, mRNA expression levels of inflammation-related genes were compared between the dipeptide groups and the Control group to which PBS was added in place of the dipeptide, and IL-1β was found to have been significantly reduced in the hydrophobic peptide fraction, thereby suggesting an effect of the dipeptides on suppression of inflammation.

Claims

[Claim 1]

An anti-inflammatory agent comprising as an active component a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 2]

The anti-inflammatory agent according to claim 1, wherein the active component is selected from the group consisting of (D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 3]

An agent for suppressing inflammatory cytokine production comprising as an active component a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-

(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 4]

The agent for suppressing inflammatory cytokine production according to claim 3, wherein the active component is selected from the group consisting of (D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 5]

An agent for suppressing IL-1 production or an agent for suppressing IL-6 production comprising as an active component a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 6]

The agent for suppressing IL-1 production or the agent for suppressing IL-6 production according to claim 5, wherein the active component is selected from the group consisting of (D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-

(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 7]

A food product composition for alleviating inflammation, comprising as an active component a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 8]

The food product composition according to claim 7, wherein the active component is selected from the group consisting of (D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 9]

A food product composition for suppressing inflammatory cytokine production, comprising as an active component a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro,

(D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 10]

The food product composition according to claim 9, wherein the active component is selected from the group consisting of (D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 11]

A food product composition for suppressing IL-1 production or a food product composition for suppressing IL-6 production, comprising as an active component a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 12]

The food product composition according to claim 11, wherein the active component is selected from the group consisting of (D)Asp-(D)Val, (D)Asp-(D)Ile, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Asp-(L)Val, (D)Asp-(L)Ile, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 13]

Use of a dipeptide for producing an anti-inflammatory agent selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 14]

A dipeptide for use in treatment of an inflammatory disease, wherein the dipeptide is selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.

[Claim 15]

A method for treating an inflammatory disease, comprising administering an effective amount of a dipeptide selected from the group consisting of (D)Ile-(D)Pro, (D)Leu-(D)Pro, (D)Pro-(D)Ile, (D)Pro-(D)Leu, (D)Val-(D)Pro, (D)Pro-(D)Val, (D)Leu-(D)Hyp, (D)Ile-

(D)Hyp, (D)Val-(D)Hyp, (D)Asp-(D)Ile, (D)Asp-(D)Val, (D)Asp-(D)Leu, (D)Asp-(D)Phe, (D)Ile-(L)Pro, (D)Leu-(L)Pro, (D)Pro-(L)Ile, (D)Pro-(L)Leu, (D)Val-(L)Pro, (D)Pro-(L)Val, (D)Leu-(L)Hyp, (D)Ile-(L)Hyp, (D)Val-(L)Hyp, (D)Asp-(L)Ile, (D)Asp-(L)Val, (D)Asp-(L)Leu, and (D)Asp-(L)Phe.