JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Occurrence of a CGRP-Like Molecule in Siki (*Centroscymnus coelolepsis*) Hydrolysate of Industrial Origin

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Fish protein hydrolysates (FPH) may have potential as bioactive components in functional foods as nutraceuticals. This study focused on the identification of calcitonin gene-related peptide (CGRP) molecules in FPH. CGRP is a neuropeptide belonging to the calcitonin/CGRP family and is known as potent arterial and venous vasodilator in humans. Hydrolysates of industrial origin were prepared from siki (*Centroscymnus coelolepsis*) heads and were analyzed for the presence of CGRP-like molecules using specific radioimmunoassays and radioreceptorassays. The biological activity of the CGRP-related molecules was assessed by their ability to stimulate the adenylate cyclase activity in rat liver membranes. They were finally purified using gel exclusion chromatography and high-performance liquid chromatography (HPLC). These molecules presented a molecular weight around 1500−2500 Da and were obtained with a purification factor of 79. The incorporation of FPH with CGRP-like molecules in functional foods could lead to the development of new useful products for health and nutrition markets.

KEYWORDS: Functional foods; siki; CGRP; protein hydrolysate; bioactive peptides

INTRODUCTION

The disposal or utilization of seafood processed waste is a critical issue for the seafood industry. For example, discarded shrimp represents more than 30 million kg of waste material per year in Norway. Such waste is either discarded in the sea or is processed into animal feed of low added value. Nonetheless, this raw material has a nutritional value that is almost equivalent to that of fish itself and has a real potential as a food ingredient (*1*). This waste constitutes an important source of biologically active molecules possessing peculiar properties and promising practical applications in various areas (including nutraceutical field that represents a rising interest). In this context, the preparation by enzymatic processes of protein hydrolysates from seafood byproducts has been extensively studied for more than 20 years. The publications on this topic relate primarily to enzymatic technology (*2*, *3*) or nutritional properties of the hydrolysates (*4*). A review by Kristinsson and Rasco (*5*) highlights the interest of the development of these technologies to produce new food ingredients, similar to those obtained by enzymatic hydrolysis from milk or plant proteins. The publications concerning the identification of molecules of therapeutic interest deal mainly with the presence of ACE-inhibitory peptides in fish protein hydrolysates (FPH) (*6*, *7*). In this field, we have previously reported the presence of calcitonin gene-related peptide (CGRP)-like molecules in several fish protein hydrolysates (*8*). CGRP is a 37-residue neuropeptide which was first described in 1982 (*9*). The structure of CGRP is strongly conserved during evolution. It is generated from the alternative splicing of the calcitonin gene, leading to the production of mRNA encoding calcitonin in thyroid C-cells or the neuropeptide CGRP in a subset of central and peripheral neurons (*10*). In humans, CGRP exerts a wide variety of biological effects on various tissues. It is known as one of the most potent arterial and venous vasodilators (*11*), with a potency 10-fold greater than the prostaglandins and 100−1000 times greater than other classic vasodilators such as acetylcholine, adenosine, 5-hydroxytryptamine, or substance P (*12*). This effect has been reported in vitro (*13*, *14*) and in vivo (*15*). In addition to its great potency, CGRP also differs from other vasodilator substances in that it has a particularly long duration of action (*12*). The capacity of CGRP to increase the rate and force of contraction of the heart (*16*) and the ability to suppress gastric acid secretion, decreasing food intake (*17*, *18*), have also been widely described. CGRP-like molecules have also been detected in nonmammalian vertebrates (*8*, *19*) and invertebrates, and nerves containing CGRP-like immunoreactivity have been described in the gastrointestinal canal of fish (*20*).

In this study, we report the characterization and purification of CGRP-like molecules from protein hydrolysates of siki (*Centroscymnus coelolepsis*) of industrial origin. The molecules were identified by specific radioimmunoassay and radiorecep-

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10.1021/jf070302l CCC: $37.00
Published on Web 06/19/2007

torassay. The CGRP-like molecules were purified using gel exclusion and high-performance liquid chromatography (HPLC). Finally, CGRP-like biological activity of the purified molecules was demonstrated by their capacity to stimulate the cAMP production in rat liver membranes, a specific target organ for CGRP. The purification of this broad-spectrum peptide and its further incorporation in functional foods would be of interest for the food industry to launch new health products on the market, seeking in this way to improve the use of seafood wastes.

MATERIALS AND METHODS

Chemicals. Human CGRP was obtained from Bachem (Weil am Rhein, Germany). Labeled hormones (specific activity: 2000 Ci/mmole) were from GE Healthcare (Chalfont St. Giles, United Kingdom). The antihuman CGRP antibody was a generous gift of Dr. A. Jullienne from Paris, France. Male Wistar rats were obtained from Janvier breeding (Le Genest sur Isle, France). The enzyme inhibitor h-αCGRP$_{8-37}$ was obtained from Sigma Chemical (St Louis, MO). Bovine serum albumin was from Sigma and was heat-inactivated before radioreceptorassays. Other chemicals were of reagent grade.

Protein Hydrolysate. Protein hydrolysate of siki (*Centroscymnus coelolepsis*) of industrial origin was prepared from cooked heads by COPALIS (Boulogne-Sur-Mer, France). Two proteases at optimum pH and temperature were consecutively used for 2 h, but more production details cannot be revealed. The enzymes were inactivated by boiling at 80 °C, and the hydrolysate was sieved to remove the bone. Then, the liquid was clarified by using a centrifuge (Westfalia separator CA220) to remove the insoluble residues. The protein hydrolysate was purified by membrane treatment: the skid of ultrafiltration was equipped with mineral membranes with a cutoff of 0.14 micrometers. The clarified hydrolysate was then deodorized by filtration with active carbon, was concentrated with a vacuum steam evaporator, and then was dried by freeze dryer.

Radioimmunoassay (RIA). Immunoreactive CGRP was measured following a previously described assay for human CGRP (*21*): in brief, an anti-CGRP antiserum at a final dilution of 1/200 000 was incubated with serial dilutions of synthetic human CGRP or siki hydrolysate. Antiserum was first incubated with tissue extracts or standard peptide for 18 h at 30 °C, and then ^{125}I labeled human CGRP was added, and the incubation continued for 24 h at 4 °C. Bound and free hormone was separated by charcoal-dextran precipitation. Control (specific antibody omitted) tubes were incubated in each assay. The hydrolysate was assayed in triplicate at multiple dilutions. Results were expressed as the percentage of initial binding, $100 \times B/B_0$, where B_0 represents the binding of labeled peptide in the absence of unlabeled hormone. Linearization of the standard curves was achieved by plotting logit ($\ln(B/B_0)/[1 - (B/B_0)]$) as a function of ln hormone or protein concentration. Only the straight lines that present a slope similar to that obtained with the standard were considered as positive, that is, containing CGRP-like molecules. The detection limit for radioimmunoassay was 10 pg of immunoreactive peptide per tube.

Calcitonin-like immunoreactivity was also quantified by a specific radioimmunoassay performed as described previously (*8*).

Liver Membrane Preparation. Liver membranes were prepared using male Wistar rats according to the method of Neville until step 11 (*22*). Proteins were quantified by the method of Lowry et al. using BSA as standard (*23*).

Radioreceptorassay (RRA). Receptor binding ability of immunoreactive molecules was developed using rat liver membranes and ^{125}I labeled human CGRP. Incubations, in a 400 μL final volume, were performed at 22 °C for 1 h (*24*). At the end of the incubation, bound and free ligand was separated by centrifugation in a solution containing 2% BSA. Each batch was tested at least with four increasing protein concentrations, and only the straight lines presenting slopes similar to that obtained with the standard hormone (10−100 pg/tube) were considered as positive. Specific activity, as it represents the quantity of CGRP-like molecules (pg) per μg of protein, was calculated. Receptor binding ability of each purified fraction (ED_{50}) was also determined and expressed as the quantity of protein (mg) that induced a 50% inhibition of the initial binding to rat liver membranes. The experiment was performed in triplicate.

Adenylate Cyclase Activity. The adenylate cyclase activity in rat liver membranes was determined by measuring the synthesis of cAMP from nonradioactive ATP as previously described (*24*). The assay was initiated by addition of membranes (12 μg) to preheat (4 min at 30 °C) assay tubes with sample or CGRP. The tubes were then incubated for 30 min at 30 °C with Hepes Tris buffer (25 mM, pH 7.4) containing 1 mM isobutylmethylxanthine (IBMX), 1.3 mM EGTA, 5 mM $MgCl_2$, 0.4 mg/mL BSA, 1 mM ATP, and 100 μM GTP. The buffer also contained 10 mM phosphocreatine and 0.8 mg/mL creatine phosphokinase as regenerating system. After incubation, the reaction was terminated by the addition of 200 μL Tris−EDTA buffer, pH 7.4, and the tubes were placed in a boiling water bath for 4 min. Cyclic AMP was quantified on 50 μL aliquots using the radioreceptor assay kit from GE Healthcare. The adenylate cyclase activity was expressed as picomoles of cAMP synthesized by 1 mg membrane protein during 30 min of incubation. The adenylate cyclase activity was also measured as above but in the presence of 1 μM αCGRP$_{8-37}$, a specific antagonist of CGRP-action (*25*). The experiment was performed in quadruplicate.

Purification of Siki Hydrolysates. Siki hydrolysate was prepurified by gel exclusion chromatography on HW 40 toyopearl column (2.5 × 33.5 cm) using ammonium acetate 0.2 M, pH 5, as eluant. The flow rate was 25.2 mL/h. The column was calibrated with the following molecular weight markers: aprotinin (6000), CGRP (3750 Da), and bacitracin (1411 Da). Aliquots were analyzed for CGRP-immunoreactivity. Immunoreactive fractions were then analyzed using CGRP radioreceptorassay. Immunoreactive and biologically active fractions were subjected to reversed-phase HPLC on a C18 prosphere column, using a linear gradient of 10−60% acetonitrile in 0.1% TFA. Flow rate was 0.9 mL/min, and fractions were collected every minute. Optical density was measured at 226 nm. Immunoreactive fractions were subjected to radioreceptorassay, and positive fractions were then repurified using the same column and a linear gradient of 13−26% acetonitrile in 0.1% TFA.

Protein Determination. The protein concentration of each analyzed fraction was quantified using the bicinchoninic acid protein assay reagent from Interchim (Montluçon, France) using BSA as the standard (*26*).

Statistical Analysis. Variance analyses (ANOVA) was performed to determine the existence of significant differences between slopes (Statgraphics plus software). A value of $p < 0.01$ was considered statistically significant. ANOVA was also used to compare the adenylate cyclase activities obtained in the different experimental conditions ($p < 0.05$).

RESULTS

1. CGRP Radioimmunoassay and Radioreceptorassay of Crude Extract. Siki hydrolysate was resuspended in distilled water at a concentration of 0.5 g/mL and was analyzed for the presence of CGRP-like molecules. Radioimmunoassay showed that the extract interacted with the antibody in the same manner as unlabeled CGRP (**Figure 1a**), according to the absence of significant differences between slopes of the straight lines obtained. Subsequent radioreceptor assay demonstrated the ability of the CGRP immunorelated peptides to interact with the CGRP receptors in rat liver membranes (**Figure 1b**). The quantity of immunoreactive CGRP-like molecules in the crude extract was 75 pg/mg of protein, and an inhibition of 50% of the initial CGRP binding to their receptors (ED_{50}) was obtained with 4.1 mg of protein. Furthermore, using a specific calcitonin radioimmunoassay, no calcitonin-like molecules could be detected in this siki hydrolysate (data not shown).

2. Molecular Sieving. Subsequently, the hydrolysate was subjected to gel filtration chromatography to separate the peptides according to their molecular weight. The different fractions were collected every 10 min and then were analyzed using CGRP-radioimmunoassay (**Figure 2a**). Five main immu-

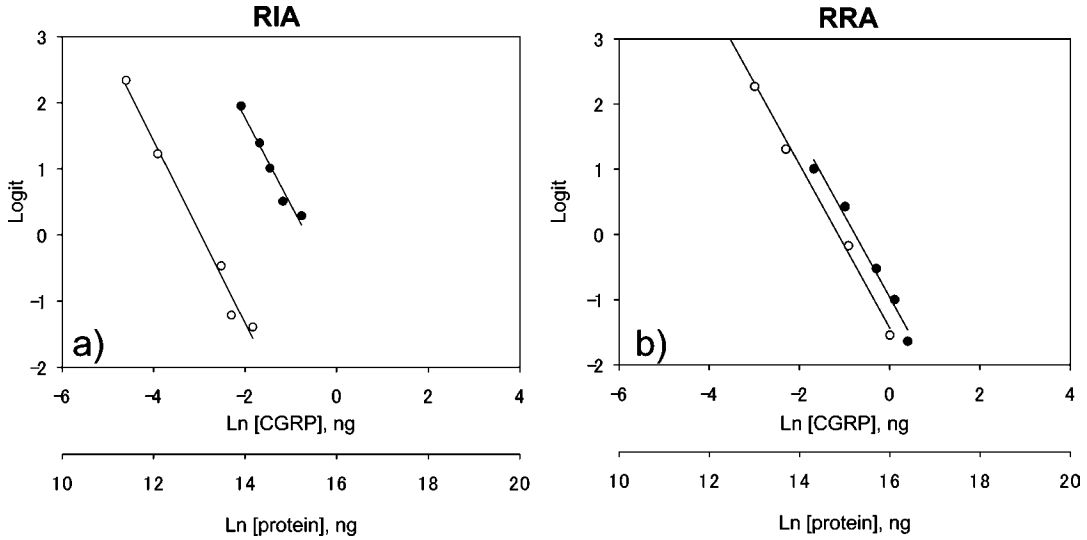

Figure 1. Radioimmunoassay and radioreceptorassay of siki hydrolysate. (**a**) RIA: Effect of increasing concentrations of siki hydrolysate (●) and unlabeled CGRP (○) on the binding of ^{125}I labeled CGRP to its antibody. Correlation coefficients for the different lines were $y = -1.38x - 4.08$ ($R^2 = 0.99$) and $y = -1.32x + 20.21$ ($R^2 = 0.97$) for unlabeled CGRP and siki hydrolysate, respectively. (**b**) RRA: Effect of increasing concentrations of siki hydrolysate on the binding of ^{125}I labeled CGRP to its receptors in rat liver membranes. Correlation coefficients for the different lines were $y = -1.24x - 1.46$ ($R^2 = 0.99$) and $y = -1.25x + 19.05$ ($R^2 = 0.98$) for unlabeled CGRP and siki hydrolysate, respectively.

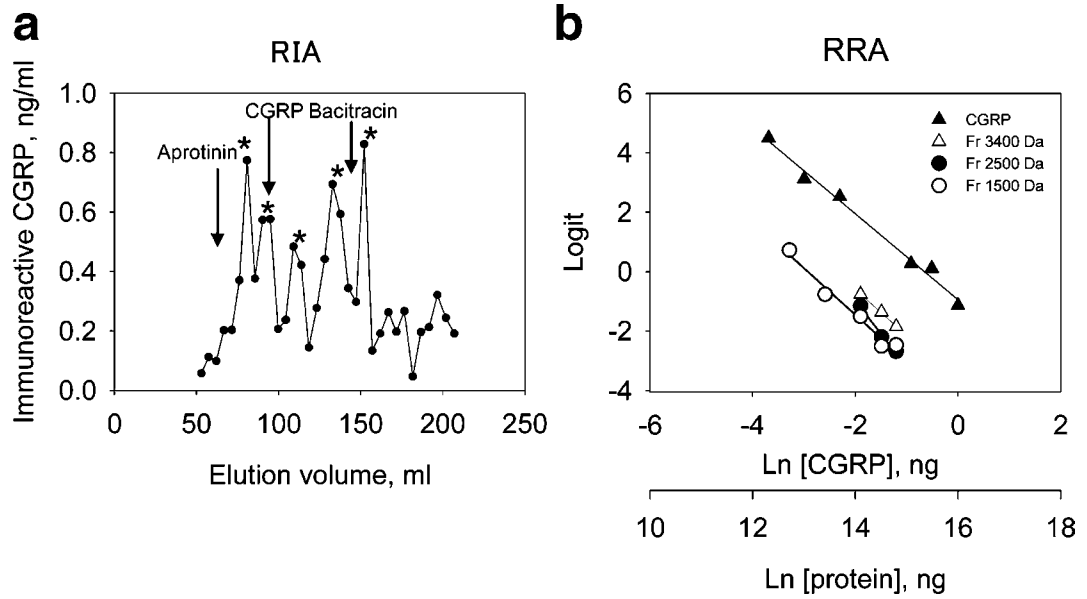

Figure 2. Gel exclusion chromatography of the siki hydrolysate. (**a**) RIA: CGRP immunoreactivity of fractions resulting of molecular sieving of siki hydrolysate on a HW Toyopearl column. (∗) Fractions used in the subsequent radioreceptorassay. (**b**) RRA: Effect of increasing concentrations of the immunoreactive fractions on the ^{125}I labeled CGRP binding to its receptor in rat liver membranes. Correlation coefficients for the different lines were $y = -1.44x - 0.94$ ($R^2 = 0.99$, CGRP), $y = -1.56x + 21.25$ ($R^2 = 1$, Fr 3400 Da), $y = -1.97x + 26.67$ ($R^2 = 1$, Fr 2500 Da), and $y = -1.57x + 20.52$ ($R^2 = 0.98$, Fr 1500 Da).

noreactive fractions were found between 75 and 150 mL of elution. These fractions contained CGRP-like molecules and corresponded to molecules of different molecular weight, around 4750, 3400, 2500, 1500, and 1000 Da. The second peak (3400 Da) had the same elution volume as CGRP. The immunoreactive fractions were further subjected to radioreceptorassay, and only three slopes were not significantly different to that obtained with unlabeled CGRP (**Figure 2b**). These slopes corresponded to fractions including molecules around 3400, 2500, and 1500 Da, which were able to interact with the CGRP receptor in rat liver membranes. The quantity of immunoreactive CGRP-like molecules was 2-fold lower in the 3400 Da fraction than in the fractions of 2500 and 1500 Da (**Table 1**). Interestingly, the quantity of proteins inducing a 50% inhibition of the CGRP binding to its receptors was lower with the smallest molecules, suggesting that these molecules (1400 Da) were the most effective in this assay (**Table 1**).

Table 1. Quantity of CGRP-Like Molecules (Mean ± Standard Deviation) in the Purified Fractions and ED_{50} Values (RRA)[a]

fraction (molecular weight)	[CGRP-like molecules], pg/mg protein	ED_{50},[b] (mg protein)
≈3400 Da	676 ± 17.62	0.82
≈2500 Da	1108 ± 168.70	0.78
≈1500 Da	1191 ± 189.30	0.48

[a] Each experiment was performed with five different dilutions in a single assay. [b] Results of ED_{50} express the quantity of proteins (mg) inducing a 50% inhibition of the CGRP-labeled binding.

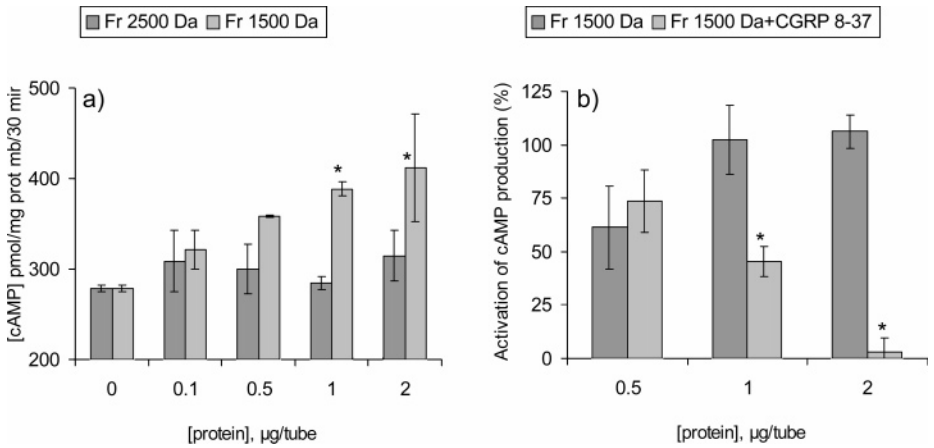

Figure 3. Adenylate cyclase activity of the purified molecules. (**a**) Effect of CGRP-immunorelated molecules obtained after gel filtration chromatography on the CGRP stimulated adenylate cyclase activity in rat liver membranes. Each bar represents the mean ± standard deviation of one experiment performed in quadruplicated. (∗) $p < 0.05$ when compared to the basal level. (**b**) Effect of 1 μM $CGRP_{8-37}$ on the stimulation of the adenylate cyclase activity mediated by the CGRP-like molecules presented in the 1500 Da fraction. Basal level of adenylate cyclase activity: 279 ± 3.83 pmol cAMP/mg membrane protein/30 min (∗) $p < 0.05$ when compared in samples with or without $CGRP_{8-37}$.

3. Adenylate Cyclase Assay. The CGRP-like biological effect of the CGRP-related molecules obtained after gel exclusion chromatography was assayed using the ability of CGRP to stimulate the adenylate cyclase activity in rat liver membranes. Only fractions containing the highest level of immunoreactive molecules were tested in this assay, that is, the 2500 and the 1500 Da fractions. Interestingly, only the immunorelated CGRP-like molecules from fraction 1500 Da were able to significantly stimulate the adenylate cyclase activity in rat liver membranes (**Figure 3a**). Increasing protein concentrations between 0.1 and 2 μg induced a dose-related stimulating activity, especially from 1 μg. With 2 μg of protein, the stimulated adenylate cyclase activity represented a 46-fold increase over the control sample. In contrast, protein concentrations (0.1−2 μg) of the 2500 fraction slightly stimulated adenylate cyclase activity, but this effect was not significant.

To demonstrate the specificity of the observed stimulation, the effect of the 1500 Da fraction on the adenylate cyclase activity was measured in the presence and the absence of 1 μM $\alpha CGRP_{8-37}$ (**Figure 3b**). The peptide $\alpha CGRP_{8-37}$ is a C-terminal fragment of human CGRP with potent antagonist properties. The cAMP production mediated by the CGRP-like molecules was significantly blocked by addition of $\alpha CGRP_{8-37}$, which interfered with the binding between the CGRP-like peptides and their specific receptors in rat liver membranes. The effect of $\alpha CGRP_{8-37}$ was assayed with increasing concentrations of 0.5−2 μg of protein. The inhibitory effect was significant with protein concentrations of 1 and 2 μg, representing an inhibition of cAMP production of 56 and 97%, respectively.

4. Purification by HPLC. To purify the CGRP-like peptides, 2 mg of each immunorelated fraction (2500 and 1500 Da) was separately subjected to high-performance liquid chromatography (HPLC). A linear gradient 10−60% acetonitrile was used for 60 min. A similar immunoreactive profile was obtained in both cases (data not shown), so these fractions (2500 and 1500 Da) were mixed together and subjected to successive HPLC. A linear gradient 10−60% acetonitrile was first used for 60 min (**Figure 4**). The immunoreactive profile of this separation showed four main peaks at elution times of 22, 26, 28, and 30 min.

These peaks were further analyzed using the CGRP radioreceptor assay (**Figure 5a**). Only fractions eluted after 28 and 30 min showed slopes similar to that obtained with unlabeled CGRP. The purification factor obtained was around 31. The concentration of CGRP-like peptides was 2.36 pg/μg of protein, which is a very high increase when compared to the value for the crude extract (75 pg/mg protein). Furthermore, 50% inhibition of the initial binding of unlabeled CGRP to its receptors was obtained with 0.19 mg of protein. This is a 22-fold lower value than that obtained with the crude extract (**Table 2**).

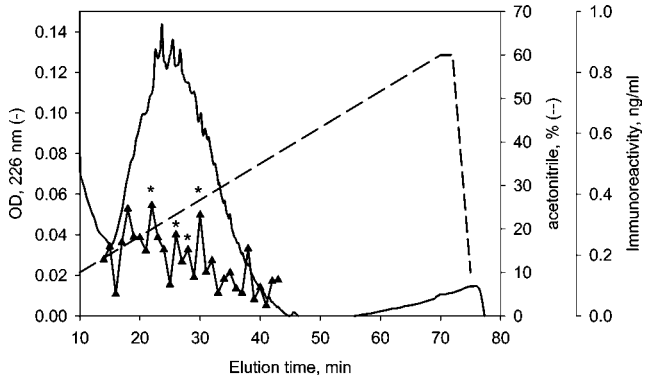

Figure 4. HPLC elution profile on C18 protein/peptide HPLC column of the immunoreactive molecules obtained after molecular sieving. A linear gradient (10–60% acetonitrile in 0.1% TFA) was used for 60 min. CGRP-like molecules (▲) were determined on an aliquot from each fraction.

The positive fractions were mixed together and were subjected to a second HPLC. A linear acetonitrile gradient (13−26%) was then used for 60 min. The elution profile is shown in **Figure 6**. CGRP-like immunoreactivity was associated with a single prominent peak. The immunoreactive fraction was also able to interact in the CGRP radioreceptor assay in a similar manner as the unlabeled CGRP (**Figure 5b**). Concentration of CGRP-like peptides increased (5.90 pg/μg), and ED_{50} noticeably decreased. Fifty percent inhibition of the initial binding of unlabeled CGRP to its receptors was observed with only 0.01 mg of protein (**Table 2**). The final ED_{50} value was 410-fold lower than that obtained with the crude extract.

DISCUSSION

In the present work, we report the occurrence of CGRP-like molecules in siki (or dogfish) hydrolysate of industrial origin. From 6930 μg of protein present in the crude extract, we obtained 8 μg of CGRP-like molecules after purification. These molecules were obtained with a purification factor of 79 and

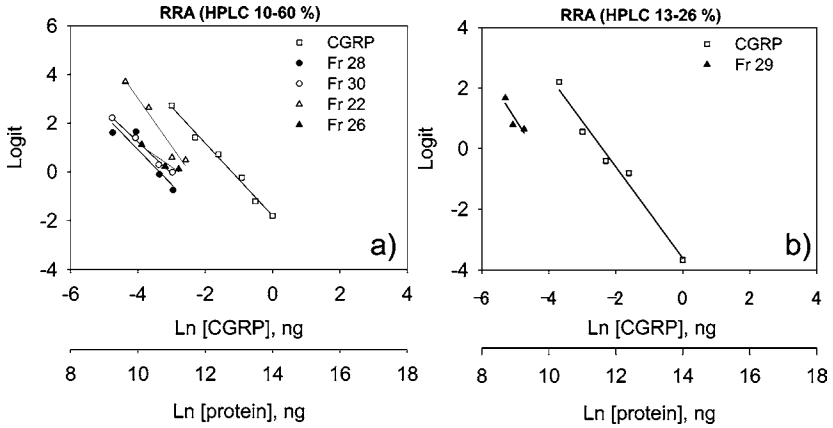

Figure 5. Radioreceptorassay of the immunoreactive fractions obtained after HPLC. (**a**) Effect of increasing concentrations of the immunoreactive fractions obtained after the first HPLC on the ^{125}I-CGRP binding to its specific receptors. Correlation coefficients for the different lines are $y = -1.49x - 1.81$ ($R^2 = 0.99$, unlabeled CGRP), $y = -1.91x + 22.18$ ($R^2 = 0.98$, fr 22), $y = -1.41x + 15.41$ ($R^2 = 0.99$, fr 26), $y = -1.43x + 15.24$ ($R^2 = 0.86$, fr 28), and $y = -1.30x + 14.22$ ($R^2 = 0.99$, fr 30). (**b**) Effect of increasing concentrations of the immunoreactive fraction obtained after HPLC 13–26% acetonitrile (fraction 29) on the ^{125}I-CGRP binding to its specific receptors. Correlation coefficients for the different lines are $y = -1.50x - 3.61$ ($R^2 = 0.98$) and $y = -1.77x + 16.91$ ($R^2 = 0.88$) for unlabeled CGRP and fraction 29, respectively.

Table 2. Purification of CGRP-Like Molecules Obtained from Siki Hydrolysates

	proteins, μg	CGRP-like immunoreactivity, pg	specific activity,[a] pg/μg protein	purification factor[b]	RRA ED_{50},[c] mg protein
crude extract	6930	520	0.075 ± 0.00		4.1
gel exclusion	1400	308	0.22 ± 0.07	2.93	1.08
HPLC 1 (10–60)	13	31	2.36 ± 0.34	31.5	0.19
HPLC 2 (13–26)	8	47	5.90 ± 0.59	78.7	0.01

[a] The specific activity (mean ± standard deviation) represents the quantity of CGRP-like molecules (pg) per μg of protein. [b] The purification factor is the ratio of the specific activity of the crude extract to that obtained at each purification step. [c] Results of ED_{50} express the quantity of proteins (mg) inducing a 50% inhibition of the CGRP-labeled binding.

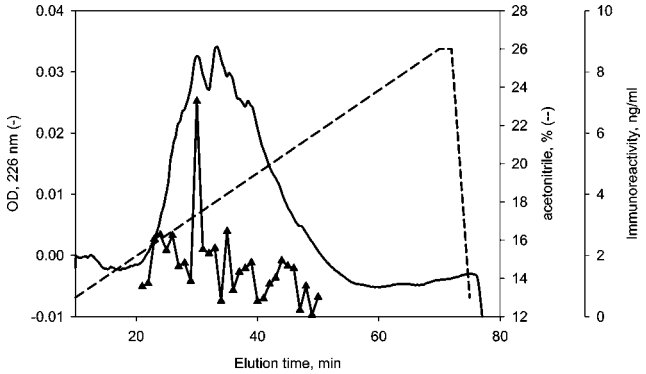

Figure 6. Second HPLC elution profile. The CGRP-like molecules obtained from the first HPLC chromatography were further purified on the same HPLC column using a linear gradient from 13 to 26% acetonitrile in 0.1% TFA for 60 min. The quantity of CGRP-like molecules (▲) was determined on an aliquot from each fraction.

presented a molecular weight around 2500−1500 Da. Apparently, these CGRP-like molecules are slightly smaller than human CGRP (3500 Da) but could possess biological activities similar to those of human CGRP. They have the ability to modulate the adenylate cyclase activity in rat liver membranes, as the neuropeptide CGRP does (*24*). In tissue preparations, it has been observed that the activation of adenylate cyclase by CGRP leads to increases in intracellular cAMP, which can exert the relaxation of smooth muscle in an underlying mechanism where cGMP and protein kinase A are implied, leading in turn to the activation of K_{ATP}^+ channels (*27*). This effect was obtained for peptide concentrations as low as 0.1 μg. In addition, we demonstrated that the increased adenylate cyclase activity observed after addition of the purified CGRP-like molecules could be reversed in the presence of $\alpha CGRP_{8-37}$, a specific antagonist of CGRP action. This specific inhibitory effect suggests that the CGRP-like molecules purified from siki hydrolysate may be more similar to αCGRP, as $\alpha CGRP_{8-37}$ can antagonize the relaxation induced by αCGRP but not by βCGRP (*28*). It might be of importance because βCGRP and αCGRP could modulate different physiological functions, βCGRP being more involved in the inhibition of gastric acid secretion in humans (*29*). It is possible that there is a close structural similarity between the purified CGRP-like molecules from siki FPH and some CGRP fragments making an interaction with the CGRP receptor possible in rat liver membranes. In fact, it has been reported that several fragments of CGRP can interact independently with the corresponding receptors. For example, the $CGRP_{8-37}$ fragment is an antagonist while $CGRP_{1-7}$ is important for efficacy (*16*).

The presence of CGRP-like molecules in FPH has also been reported in cod hydrolysates from heads, stomach, and viscera (*8*) and in sardine hydrolysate (*30*). In these sardine hydrolysates performed on a lab scale, the CGRP-like molecules identified acted like inhibitors of CGRP action and are reported to have molecular weight around 6000 Da, substantially higher than that reported here for siki hydrolysate of industrial origin.

The broad variety of biological effects of CGRP possibly mediated by several CGRP receptor subtypes implies that several in vivo experiments may be performed to determine the real effects of these obtained CGRP-like molecules in vertebrates. In this field, some studies have described the beneficial effects of short-term or prolonged infusions of CGRP in patients to increase cardiac output and to decrease the blood pressure without changes in heart rate (*31*, *32*). The capacity to exert gastric acid protection by decreasing acid secretion and by increasing blood flow has also been documented (*18*, *29*, *33*) as well as its role in the processing of auditive information and facilitation of learning and memory processing (*28*, *34*, *35*). All

in vivo and in vitro assays performed with CGRP involve the promising incorporation of the CGRP-like molecules into foods, which could lead to the development of a new useful product for health and nutrition-related markets. By inducing vasodilation, the incorporation of CGRP-like molecules in functional foods might be an important defense mechanism against the serious consequences of increased plasma volume including edema, hypertension, and increased cardiac workload. Functional products with CGRP-like molecules might also have therapeutic potential in the treatment of another pathologies such as obesity or gastric ulcer.

In summary, it has been demonstrated that the industrial production of protein hydrolysate of siki (*Centroscymnus coelolepsis*) could generate the production of CGRP related molecules. These molecules are able to stimulate the adenylate cyclase activity in rat liver membranes. The protein hydrolysate could exert a positive effect on humans, and its incorporation in new functional foods should be taken into account. Further work will concern the sequence and the in vivo tests of the purified peptide.

ACKNOWLEDGMENT

We thank Mrs. Andrea Bullock for carefully reviewing the manuscript.

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Received for review February 2, 2007. Revised manuscript received April 25, 2007. Accepted May 11, 2007. This work was supported by a grant from the European Community (Seafoodplus CT-2004-506359). A part of this work was presented at the Second Joint TransAtlantic Fisheries Technology conference held in Quebec Canada (October 2006). Oscar Martinez Alvarez is under a Spanish fellowship of the Ministry of Education and Science.

JF070302L