Biochemical and Biophysical Research Communications 386 (2009) 153–158

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Ghrelin *O*-acyltransferase (GOAT) has a preference for *n*-hexanoyl-CoA over *n*-octanoyl-CoA as an acyl donor

Hideko Ohgusu, Kaori Shirouzu, Yuki Nakamura, Yoshiki Nakashima, Takanori Ida, Takahiro Sato, Masayasu Kojima *

Molecular Genetics, Institute of Life Science, Kurume University, Hyakunenkohen 1-1, Kurume, Fukuoka 839-0864, Japan

ARTICLE INFO

Article history:
Received 28 May 2009
Available online 6 June 2009

Keywords:
Ghrelin
Ghrelin *O*-acyltransferase (GOAT)
Acyl-modification
n-Octanoyl-CoA
n-Hexanoyl-CoA

ABSTRACT

Ghrelin is a peptide hormone in which serine 3 is modified by *n*-octanoic acid through GOAT (ghrelin *O*-acyltransferase). However, the enzymological properties of GOAT remain to be elucidated. We analyzed the *in vitro* activity of GOAT using the recombinant enzyme. Unexpectedly, although the main active form of ghrelin is modified by *n*-octanoic acid, GOAT had a strong preference for *n*-hexanoyl-CoA over *n*-octanoyl-CoA as an acyl donor. Moreover, a four-amino acid peptide derived from the N-terminal sequence of ghrelin can be modified by GOAT, indicating that these four amino acids constitute the core motif for substrate recognition by the enzyme.

Introduction

Ghrelin is a peptide hormone that is secreted from the stomach in response to hunger and starvation, and serves as a peripheral orexigenic signal transmitting information to the brain and encouraging consumption of food [1–4].

Ghrelin has been identified in almost all vertebrate species examined, in both mammalian and non-mammalian species, e.g., frogs, birds, and fish [5]. In its active form, ghrelin bears an acyl-modification at the third amino acid (serine in most species; threonine in the frog). This acyl-modification is necessary for ghrelin to bind to the ghrelin receptor and in order for it to exert biological activity [1]. The structure of ghrelin, particularly that of the acyl-modified regions, is highly conserved throughout all vertebrate species [6]. The fatty acyl group that is used for the modification of ghrelin is primarily *n*-ocatnoic acid. In order to elucidate the mechanism of the unique acyl-modification seen in ghrelin, investigations characterizing the putative ghrelin Ser *O*-acyltransferase are required.

In 2008 Yang et al. and Gutierrez et al. independently reported an enzyme, named GOAT for ghrelin *O*-acyltransferase, which catalyzed *n*-octanoyl modification of ghrelin in cultured cells [7,8]. GOAT mRNA is highly expressed in stomach and GOAT knockout mice lack octanoylated ghrelin, consistent with the idea that GOAT is the acyltransferase required for the *n*-octanoyl modification of ghrelin [7,8]. Moreover, Sataka et al. demonstrated co-localization of GOAT and ghrelin in the mouse gastric oxyntic mucosa [9]. In addition, Yang et al. reported the first characterization of *in vitro* GOAT activity by using membrane fraction from GOAT expressing insect cell [10]. They showed that GOAT transfers [^{3}H]octanoyl group to not only pro-ghrelin but also to a pentapeptide, which contains only the N-terminal five amino acids of ghrelin. Moreover, GOAT activity was shown to be inhibited by an octanoylated ghrelin pentapeptide.

We established *in vitro* assay systems of GOAT to analyze the molecular forms of acyl-modified ghrelin and quantify produced acyl-modified ghrelin by a combination of reverse-phase HPLC (RP-HPLC) and ghrelin specific radioimmunoassay systems. Here we enzymologically characterized GOAT, and determined parameters including optimal pH, optimal temperature, substrate peptide lengths, and acyl donor preferences. Unexpectedly, we found that GOAT had a strong preference for *n*-hexanoyl-CoA over *n*-octanoyl-CoA as an acyl donor, although the main active form of ghrelin is modified by *n*-octanoic acid.

Materials and methods

Construction of stable GOAT expressing cell. Mouse GOAT cDNA was amplified by PCR using the following primer pairs: sense, 5′-TCAA GCTTAGGATGGATTGGCTCCAGCTCTTTTTTCTGCATCCTTTATC-3′, containing a HindIII site; antisense 5′-GACTCGAGTCAGTTACGTTTGT

Abbreviations: GOAT, ghrelin *O*-acyltransferase; CHO, chinese hamster ovary; CHAPS, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulphonate.

* Corresponding author. Fax: +81 942 31 5212.
E-mail address: mkojima@lsi.kurume-u.ac.jp (M. Kojima).

0006-291X/$ - see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2009.06.001

CTTTTCTCTCCGCTAACAG-3′, containing a XhoI site. GOAT cDNA was amplified from total stomach cDNA using Pyrobest DNA polymerase (Takara Bio Inc., Ohtsu, Japan) and inserted into pcDNA3.1 vector at the HindIII–XhoI site. CHO cells were transfected of GOAT-pcDNA3.1 vector and cultured in α-MEM medium with G418 (1 mg/ml) for stable cell selection. In the cells that grew in the presence of G418, expression of GOAT mRNA was confirmed by RT-PCR, using the primers described above. The cells that showed the highest expression level of GOAT were used for further experiments.

GOAT enzyme preparation. The GOAT-expressing CHO cells were cultured to 80–90% confluence in twelve cultured plates (100 mm/Tissue Culture Dish, IWAKI, Tokyo, Japan). Cells were harvested, lysed using a Teflon homogenizer in extraction buffer [100 mM Tris–HCl (pH 7.4) containing 1 mM PMSF (phenylmethylsulfonyl fluoride), 0.8 nM aprotinin, 15 μM E-64, 20 μM leupeptin, 50 μM bestatin and 10 μM pepstatin A]. The homogenate was cleared by centrifuged at 800*g* for 5 min; the resultant supernatant was further centrifuged at 100,000*g* for 1 h. The pellet was resuspended in the same extraction buffer and stored at −80 °C.

GOAT enzyme assay. The standard assay condition for ghrelin *n*-octanoyl modification contains the following: 200 μl of 50 mM Tris–HCl (pH 7.4), 0.5 μM rat des-acyl ghrelin, 10 μM *n*-octanoyl-CoA (Sigma–Aldrich Co., St. Louis, MO), 0.1% CHAPS, and 1.0 μg of membrane protein preparation from GOAT expressing cells. Rat des-acyl ghrelin was synthesized using the Fmoc solid-phase method on a peptide synthesizer (433A; Applied Biosystems, Foster City, CA), then purified by RP-HPLC. The reaction was initiated by adding the enzyme solution, and incubated at 37 °C for 30 min. The reaction was stopped by adding 20 μl 1 N HCl and stored at −30 °C until ghrelin concentration could be measured.

ELISA of ghrelin. Active ghrelin ELISA Kit (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan) was used for measuring *n*-octanoyl ghrelin. Des-acyl ghrelin ELISA Kit (Mitsubishi Kagaku Iatron, Inc., Tokyo, Japan) was used for the measurement of des-acyl ghrelin.

RIA of ghrelin. RIAs specific for ghrelin were performed as previously described [11]. All assays were performed in duplicate. The anti-rat ghrelin (1–11) antiserum, which specifically recognizes the *n*-octanoylated portion of ghrelin but does not recognize des-acyl ghrelin. Anti-rat ghrelin (13–28) antiserum equally recognizes both des-acyl and all acylated forms of ghrelin peptide, including *n*-hexanoyl, *n*-octanoyl, *n*-decanoyl, *n*-lauroyl, *n*-myristoyl, and *n*-palmitoyl ghrelins. Throughout the following sections, the RIA system using the antiserum raised against the N-terminal fragment of rat ghrelin (1–11) is termed N-RIA, whereas the RIA system using the antiserum recognizing the C-terminal fragment (13–28) is termed C-RIA.

HPLC analysis of acyl-modified ghrelin. The reaction products were loaded onto Sep-Pak C18 (Waters, Milford, MA), and pre-equilibrated in 10% acetonitrile/0.1% trifluoroacetic acid. The Sep-Pak cartridge was washed with 10% CH_3CN/0.1% trifluoroacetic acid, and the peptide fraction was eluted in 60% CH_3CN/0.1% trifluoroacetic acid. The eluate was lyophilized and separated by RP-HPLC using a μBondasphere C18 (3.9 × 150 mm; Waters) column. A linear gradient of CH_3CN from 10% to 60% in 0.1% TFA served as the solvent system, using a flow rate of 1 ml/min for 40 min. One 500-μl fraction was collected every 30 s. Each fraction was lyophilized and subjected to RIAs or ELISAs for ghrelin.

Results

Preparation of GOAT

We prepared cell homogenates from the GOAT-expressing CHO cells, and isolated the membrane fraction by sequential centrifugation. The subcellular fractionation procedure yielded specific ghrelin *n*-octanoyl transferase activity in the 100,000*g* pellet, but not in the supernatant fraction (Fig. 1A). Control CHO cells had no GOAT activity (Fig. 1A).

We next examined the effects of detergents on GOAT activity. We found that treatment of the membrane fraction with CHAPS or Tween 80 retains GOAT activity (Fig. 1B). In contrast, treatments with other six detergents we tested, Triton X100, Lubrol, NP40, Brij96v, Triton X45, and NP9, attenuated or abolished the GOAT activity. These results indicate that CHAPS and Tween 80 stabilize the conformation of GOAT, and are useful for the solubilization of GOAT.

The reaction rate was linear at protein concentration up to 2.0 μg in reaction mixture (Fig. 1C). Fig. 1D plots the production of *n*-octanoyl ghrelin against time of incubation. We found that using 1.0 μg of membrane protein preparation from GOAT expressing cells, the reaction rate was linear for at least 120 min.

Thus, the standard assay condition we used was 30 min incubation time and contained 1.0 μg of the membrane protein.

Acyl donors for GOAT enzyme reaction

We reported previously that ingested medium-chain fatty acids are used directly for acyl-modification of ghrelin [12]. However, most acyltransferases use an acyl-CoA as an acyl donor. Therefore, we attempted to determine whether free fatty acid or acyl-CoA which is the acyl donor for GOAT. We found that *n*-octanoyl ghrelin is produced when *n*-octanoyl-CoA is used for an acyl donor (data not shown). In contrast, *n*-octanoyl ghrelin is not synthesized when *n*-octanoic acid is used for an acyl donor. Moreover, co-incubation of both *n*-octanoic acid and CoA did not produce *n*-octanoyl ghrelin. We therefore conclude that *n*-octanoyl-CoA is an acyl donor for ghrelin.

Characterization of modified ghrelin peptide produced in the GOAT reaction

We analyzed the molecular forms of ghrelin in the *in vitro* GOAT reaction to confirm that the produced ghrelin peptide was actually modified by *n*-octanoyl acid. Supplementary figure shows the HPLC analysis of GOAT reaction products. Each fraction was measured by active ghrelin ELISA, which specifically reacts only with *n*-octanoyl ghrelin (1–28). We detected *n*-octanoyl ghrelin immunoreactivity at fraction 42, which is the same elution position as that of standard *n*-octanoyl ghrelin (elution time, 20.5–21.0 min). Thus, ghrelin peptide produced in the GOAT enzyme reaction is definitely *n*-octanoyl ghrelin (1–28), the major endogenous form of ghrelin both in the stomach and plasma [11].

Optimal temperature and pH of GOAT activity and inhibitory effects of iron and copper

The optimal GOAT reaction temperature is 37–50 °C (Fig. 2A). GOAT still retains some enzyme activity at 55 °C. The enzyme activity was abolished over 60 °C.

We also determined the specific activity of GOAT over a range of pH values (Fig. 2B). The optimal pH for maximal specific activity was observed at pH 7.0–7.5, which was the similar result with that by Yang et al. [10]. The specific activity dropped off rapidly below pH 6.5 and above pH 8.5.

Fe^{3+} and Cu^{2+} potently inhibited GOAT activity (Fig. 2C and D). The GOAT activities was completely blocked over 5 mM Fe^{3+} and 0.5 mM Cu^{2+}. EDTA and EGTA had no effect on GOAT activity, indicating that the enzyme has no absolute requirement for cations.

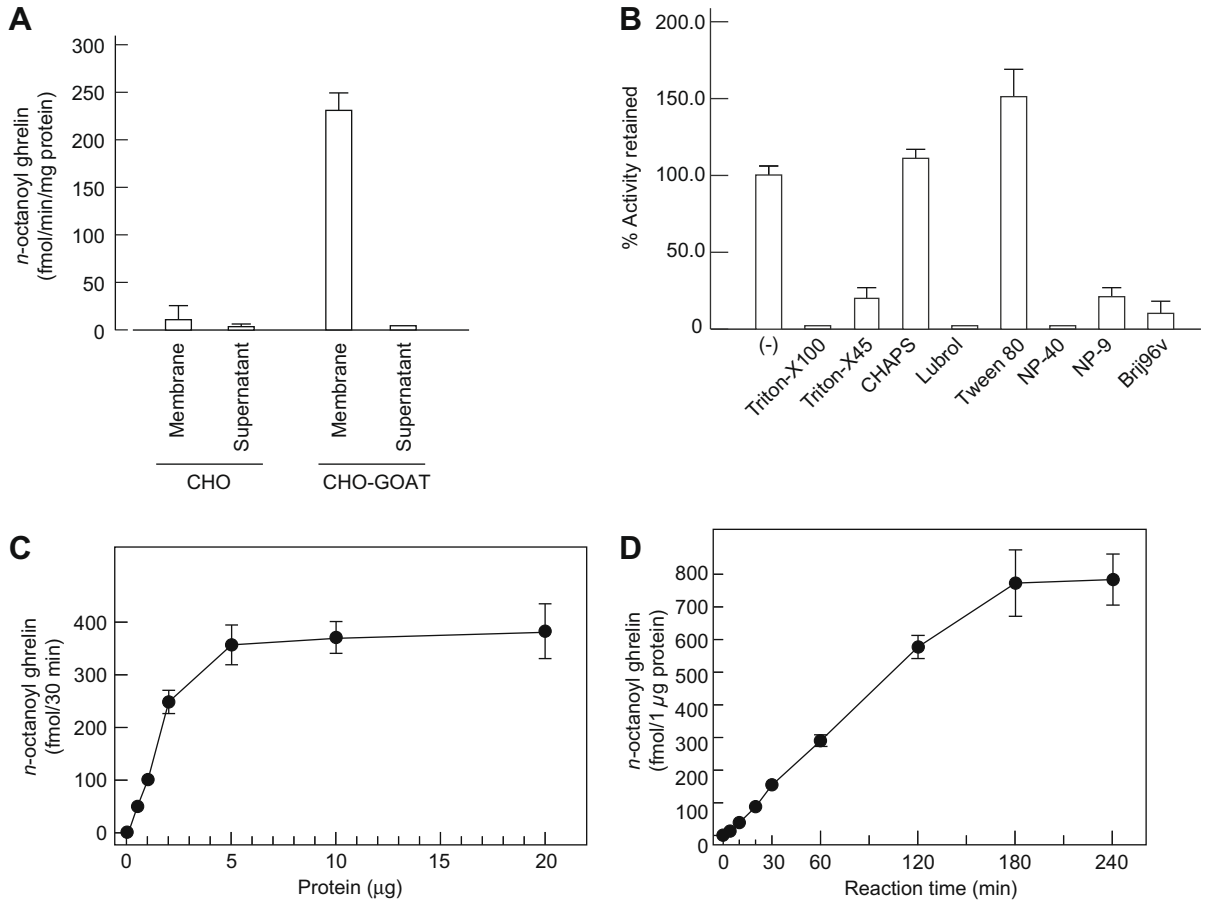

Fig. 1. Subcellular localization of GOAT activity and effects of detergents, incubation time and protein concentration on GOAT activity. (A) Wild-type CHO and GOAT-expressing CHO (CHO–GOAT) cells were collected and separated by centrifugation to obtain membrane fractions (100,000*g*). Ghrelin *n*-octanoyl modification reaction was performed using the standard assay conditions described in Experimental Procedures. *n*-Octanoyl ghrelin concentration was measured using the active ghrelin ELISA kit. (B) Effects of detergents on GOAT activity. Crude membranes from GOAT-expressing CHO cells were solubilized in 1% of the indicated detergent for 15 min at 37 °C and the *n*-octanoyl transferase reaction was performed under standard assay solution containing 0.1% of the indicated detergent. (C) Effects of incubation time and (D) protein concentration on GOAT activity. GOAT activity was measured under standard conditions, except for the parameters being examined. Produced *n*-octanoyl ghrelin was measured by active ghrelin ELISA kit. Results of (A–D) are expressed as the means ± SD ($n = 3$).

Substrate peptide lengths and GOAT activity

We next examined the substrate peptide lengths that were acyl-modified by GOAT. The synthetic peptide substrates we used in the reaction were derived from the N-terminal sequence of mammalian ghrelin. The length of these substrates (4–8 amino acids) was shorter than that of des-acyl ghrelin, and the C-termini of the substrate peptides had α-amide structures. N-RIA for ghrelin was used for the detection of *n*-octanoyl modified peptides, because N-RIA specifically recognizes the *n*-octanoyl modified part of ghrelin.

Arrows in Fig. 3(A–F) show the HPLC retention times of the five synthetic ghrelin-derived substrates. The retention times of the reaction products measured by N-RIA were increased in all reactions with the peptide substrates, regardless of length (Fig. 3A–E). The retention times of the products were at 23.5–24.0 min (GSSFLSPK-NH_2), 25.0–25.5 min (GSSFLSP-NH_2), 23.0–23.5 min (GSSFLK-NH_2), 26.0–26.5 min (GSSFL-NH_2), and 23.5–24.5 min (GSSF-NH_2). Synthetic *n*-octanoyl modified 8-amino acid peptide, GSS(C8:0)FLSPK-NH_2, had the same retention time as that of the GOAT-modified GSSFLSPK-NH_2 product (data not shown).

Immunoreactive-ghrelin concentration in the reactions revealed that the longer 8-amino acid peptide is acylated to nearly 100-fold higher levels than the shorter 4-amino acid peptide, and 4-fold higher than the 5-amino acid peptide. Because the produced amount of GOAT-treated 4-amino acid peptide (GSSF-NH_2) was very low (Fig. 3E), it may be suspected that *n*-octanoyl modification on the 4-amino acid peptide was not a GOAT specific reaction but a non-enzymatic reaction. However, we found that without GOAT enzyme solution there is no peptide product at 23.5–24.5 min (Fig. 3F).

These results indicate that these five synthetic peptides served as the substrates of GOAT and were modified by *n*-octanoic acid. Thus, a peptide as short as four amino acid constitutes the core motif for substrate recognition by GOAT.

Effect of acyl donors on GOAT activity

To determine whether GOAT exclusively utilizes *n*-octanoyl-CoA as an acyl donor, we next analyzed the reactions of the recombinant GOAT enzyme against several *n*-acyl-CoAs, including *n*-hexanoyl-CoA, *n*-decanoyl-CoA, *n*-palmitoyl-CoA, and *n*-myristoyl-CoA. We found that GOAT can modify des-acyl ghrelin peptide with not only *n*-octanoyl CoA, but also with other medium chain acyl acids, such as *n*-hexanoyl-CoA (Fig. 4A) and *n*-decanoyl-CoA. In contrast, acyl-modified products resulted from the reactions with long-chain fatty acids should be very low and we could not detected in our reaction condition.

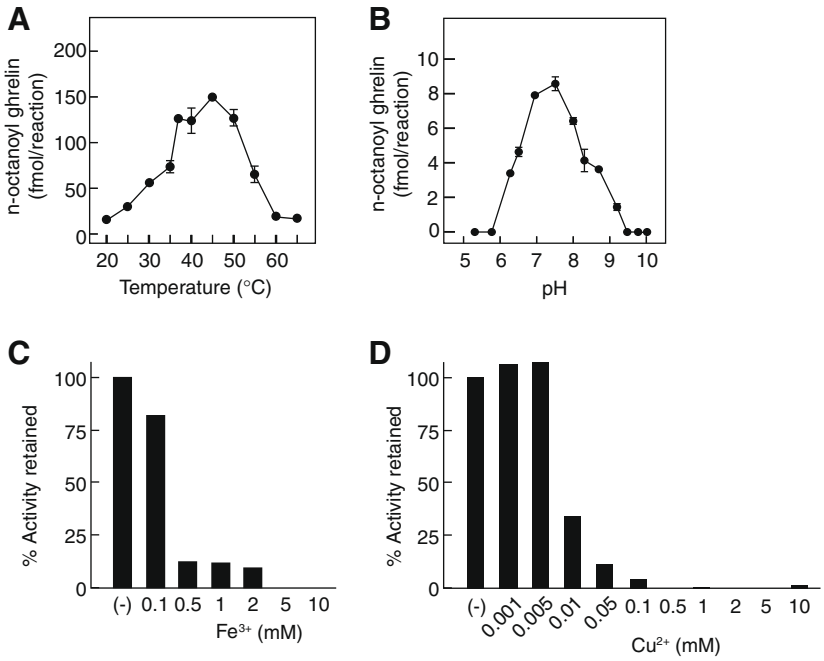

Fig. 2. Optimal temperature and pH of GOAT activity and effects of cations. *n*-Octanoyl ghrelin production activity was measured using standard assay conditions. *n*-Octanoyl ghrelin concentrations were measured by active ghrelin ELISA Kit. Results are expressed as the means ± SD ($n = 3$). (A) Temperature dependence of GOAT activity. (B) pH dependence of GOAT activity. The following buffers were used: 50 mM MES (pH 5–7), Tris–HCl (pH 7.5–8.5), and $NaHCO_3$ (pH 9–10). (C and D) Enzyme activity was measured under standard assay conditions, except for inclusion of cations. The cations added were (C) $FeCl_3$ and (D) $CuCl_2$. Activity is expressed as the percent activity retained after cation treatment as compared with standard assay conditions. Data are the average of two independent experiments.

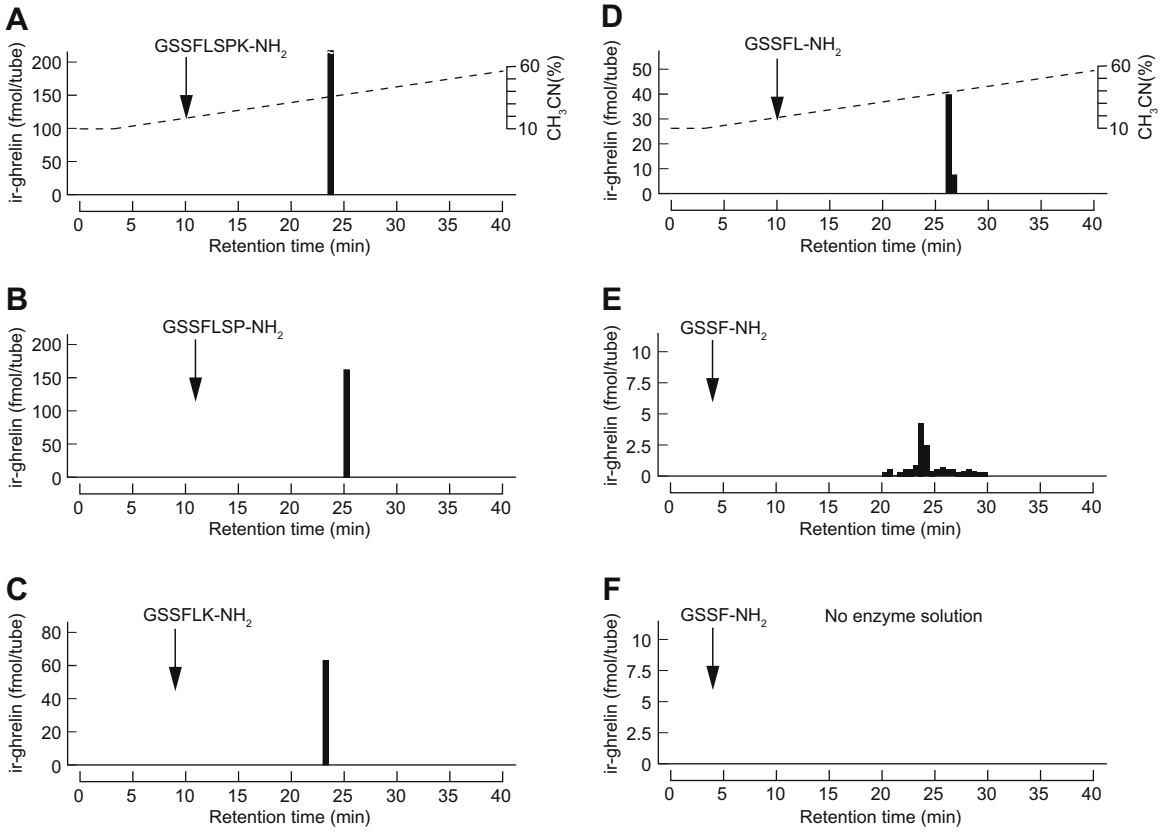

Fig. 3. Analysis of GOAT substrate specificity. (A–E) HPLC analyses of GOAT reaction products when (A) GSSFLSPK-NH_2, (B) GSSFLSP-NH_2, (C) GSSFLK-NH_2, (D) GSSFL-NH_2, and (E) GSSF-NH_2 were used as substrates. (F) HPLC analysis of GSSF-NH_2 substrate reaction without GOAT enzyme solution. Reaction products were subjected to HPLC and each fraction was assayed for immunoreactive *n*-octanoyl ghrelin by N-RIA. The eluted positions of peptide substrates are indicated by arrows.

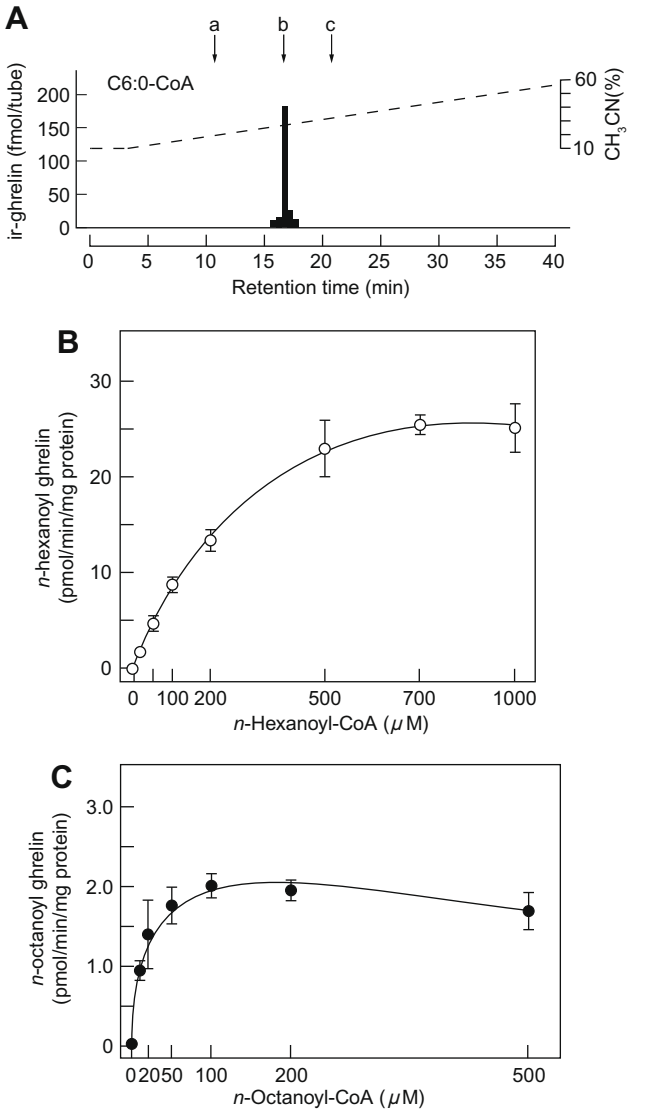

Fig. 4. Acyl-CoA specificity of GOAT. (A) Acyl-CoA specificity of GOAT toward *n*-hexanoyl-CoA (C6:0) was analyzed by incubating 0.5 μM rat des-acyl ghrelin in the presence of GOAT enzyme solution. Two reactions for *n*-hexanoyl-CoA were performed; these reactions were pooled for HPLC analyses. The reaction products were subjected to HPLC to separate acyl-modified ghrelin from des-acyl ghrelin and each fraction was assayed for immunoreactive ghrelin by ghrelin C-RIA, which recognizes the C-terminal portion of ghrelin peptide and is able to detect acyl-modified ghrelins with various length of acyl acids. The arrows indicate the eluted positions of (a) des-acyl ghrelin, (b) *n*-hexanoyl ghrelin and (c) *n*-octanoyl ghrelin. (B and C) Kinetic studies of the recombinant GOAT. GOAT assays were performed by incubating increasing concentrations of (B) *n*-hexanoyl-CoA (C6:0) and (C) *n*-octanoyl-CoA (C8:0) under the same assay conditions. Results are expressed as the means ± SD ($n = 3$). The concentrations of *n*-hexanoyl ghrelin and *n*-octanoyl ghrelin were measured using ghrelin C-RIA after HPLC. K_m values were calculated from these plot data.

We then conducted kinetic studies using des-acyl ghrelin and three medium-chain acyl-CoAs as the acyl donor: *n*-hexanoyl-CoA (Fig. 4B), *n*-octanoyl-CoA (Fig. 4C) and *n*-decanoyl-CoA (data not shown). Increased acyl-CoA concentrations resulted in increased GOAT activity. The order of the activities as evaluated by V_{max}/K_m is *n*-hexanoyl-CoA > *n*-octanoyl-CoA > *n*-decanoyl-CoA. The K_m values of *n*-hexanoyl-CoA and *n*-octanoyl-CoA were 294 and 13.6 μM, respectively. We could not calculate the K_m values of *n*-decanoyl-CoA, because the concentration of *n*-decanoyl ghrelin produced in this assay system was very low.

Discussion

Ghrelin is a peptide hormone in which the third amino acid, usually a serine but in some species a threonine, is modified by a fatty acid, primarily by *n*-octanoic acid; this modification is essential for ghrelin's activity [1,6]. Thus, the enzyme that catalyzes the acyl-modification of ghrelin is important for the regulation of ghrelin's activities, including growth hormone secretion, appetite stimulation and metabolic functions. The enzyme had not been identified until the discovery of GOAT, ghrelin *O*-acyltransferase, which belongs to the membrane-bound *O*-acyltransferase (MBOAT) family [7,8,13]. Here, we confirmed that GOAT definitely catalyzes the acyl-modification of ghrelin *in vitro*.

Our results revealed that GOAT acylates not only 28-amino acid des-acyl ghrelin but also short ghrelin-derived peptides. Yang et al. reported the *n*-octanoyl transfer by GOAT to the pentapeptide GSSFL-NH_2, which was the same pentapeptide we used in the substrate specificity experiments [10]. We found that a ghrelin derived peptide as short as four amino acids can be acyl-modified by GOAT. Thus, it is likely that GOAT recognizes a N-terminal four amino acids motif within the intact ghrelin peptide.

Unexpectedly, GOAT prefers *n*-hexanoyl-CoA over *n*-octanoyl-CoA as the acyl donor. However, the concentration of *n*-hexanoyl ghrelin in the mouse stomach is very low, compared with that of *n*-octanoyl ghrelin. Our previous report displays that ingestion of glyceryl trihexanoate drastically stimulates the production of *n*-hexanoyl ghrelin in the stomach [12]. This fact supports our observation that GOAT prefers *n*-hexanoyl-CoA as an acyl donor. Thus, we speculate that the content of *n*-hexanoyl-CoA in the stomach may be lower than that of *n*-octanoyl-CoA, and these concentration difference may affect the production and concentration of various acyl-modified ghrelins.

In summary, we examined the enzymological properties of GOAT, a ghrelin specific medium-chain acyltransferase. Because acyl-modification of ghrelin is necessary for its activity, the regulation of GOAT activity affects the physiological functions of ghrelin, in particular, appetite regulation. Thus, GOAT may be a therapeutic target for eating disorders or other metabolic diseases.

Acknowledgments

We thank Y. Hayashi and Y. Kitajima (Asubio Pharma Co., Ltd., Japan) for providing us with synthetic ghrelin derived peptides. We also thank H. Hosoda, K. Kangawa (National Cardiovascular Center, Japan) and M. Noguchi (Kurume University) for their advice. This study was supported by Grant-in-Aids for Scientific Research (B) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, Research on Measures for Intractable Diseases from the Health and Labour Sciences Research Grants, and by Program for Promotion of Basic and Applied Researches for Innovations in Bio-oriented Industry.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2009.06.001.

References

[1] M. Kojima, H. Hosoda, Y. Date, M. Nakazato, H. Matsuo, K. Kangawa, Ghrelin is a growth-hormone-releasing acylated peptide from stomach, Nature 402 (1999) 656–660.
[2] M. Nakazato, N. Murakami, Y. Date, M. Kojima, H. Matsuo, K. Kangawa, S. Matsukura, A role for ghrelin in the central regulation of feeding, Nature 409 (2001) 194–198.
[3] M. Tschop, D.L. Smiley, M.L. Heiman, Ghrelin induces adiposity in rodents, Nature 407 (2000) 908–913.

[4] A.M. Wren, C.J. Small, H.L. Ward, K.G. Murphy, C.L. Dakin, S. Taheri, A.R. Kennedy, G.H. Roberts, D.G. Morgan, M.A. Ghatei, S.R. Bloom, The novel hypothalamic peptide ghrelin stimulates food intake and growth hormone secretion, Endocrinology 141 (2000) 4325–4328.
[5] M. Kojima, T. Ida, T. Sato, Structure of mammalian and nonmammalian ghrelins, Vitam. Horm. 77 (2008) 31–46.
[6] M. Kojima, K. Kangawa, Ghrelin: structure and function, Physiol. Rev. 85 (2005) 495–522.
[7] J.A. Gutierrez, P.J. Solenberg, D.R. Perkins, J.A. Willency, M.D. Knierman, Z. Jin, D.R. Witcher, S. Luo, J.E. Onyia, J.E. Hale, Ghrelin octanoylation mediated by an orphan lipid transferase, Proc. Natl. Acad. Sci. USA 105 (2008) 6320–6325.
[8] J. Yang, M.S. Brown, G. Liang, N.V. Grishin, J.L. Goldstein, Identification of the acyltransferase that octanoylates ghrelin, an appetite-stimulating peptide hormone, Cell 132 (2008) 387–396.
[9] I. Sakata, J. Yang, C. Lee, S. Osborne-Lawrence, S. Rovinsky, J.K. Elmquist, J. Zigman, Co-localization of ghrelin *O*-acyltransferase (GOAT) and ghrelin in gastric mucosal cells, Am. J. Physiol. Endocrinol. Metab. (2009).
[10] J. Yang, T.J. Zhao, J.L. Goldstein, M.S. Brown, Inhibition of ghrelin O-acyltransferase (GOAT) by octanoylated pentapeptides, Proc. Natl. Acad. Sci. USA 105 (2008) 10750–10755.
[11] H. Hosoda, M. Kojima, H. Matsuo, K. Kangawa, Ghrelin and des-acyl ghrelin: two major forms of rat ghrelin peptide in gastrointestinal tissue, Biochem. Biophys. Res. Commun. 279 (2000) 909–913.
[12] Y. Nishi, H. Hiejima, H. Hosoda, H. Kaiya, K. Mori, Y. Fukue, T. Yanase, H. Nawata, K. Kangawa, M. Kojima, Ingested medium-chain fatty acids are directly utilized for the acyl modification of ghrelin, Endocrinology 146 (2005) 2255–2264.
[13] K. Hofmann, A superfamily of membrane-bound *O*-acyltransferases with implications for wnt signaling, Trends Biochem. Sci. 25 (2000) 111–112.