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Interaction of starfish gonadotropin with its receptor: Effect of chimeric relaxin-like gonad-stimulating peptides

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ABSTRACT

A relaxin-like gonad-stimulating peptide (RGP) of starfish *Patiria* (*Asterina*) *pectinifera* is the first identified invertebrate gonadotropin for final gamete maturation. Recently, we found three orthologs of RGP in the class Asteroida; PpeRGP in *P. pectinifera*, AamRGP in *Asterias amurensis*, and AjaRGP in *Aphelasterias japonica*. In this study, nine kinds of RGP derivatives with exchanged each A- and B-chain were synthesized chemically to analyze the interaction of RGP with its receptor. Among these RGP derivatives, PpeRGP and its chimeric RGPs with B-chains from AamRGP or AjaRGP could induce oocyte maturation and ovulation in *P. pectinifera* ovaries. In contrast, other RGP derivatives were failed to induce spawning in *P. pectinifera* ovaries. Circular dichroism spectra of PpeRGP were similar to those of chimeric RGPs with the B-chains from AamRGP or AjaRGP. Furthermore, the predicted three-dimensional structure models of the B-chains from RGP derivatives have almost the same conformation. These findings suggest that the B-chain of PpeRGP is involved in binding to its receptor. Thus, it is likely that the A-chain of AamRGP or AjaRGP disturbs the binding of the PpeRGP B-chain to its receptor.

1. Introduction

In most starfish, oocytes in a ripe ovary remain arrested at the end of the first prophase stage of meiosis. Resumption of meiosis is controlled by hormonal action. Chaet and McConnaughy (1959) first reported that a hot water extract of *Asterias forbesi* nerves could induce the shedding of gametes when injected into the coelomic cavity of ripe animals. The active substance contained in the nerve extract was named gonad-stimulating substance (GSS) (Kanatani and Shirai, 1967, 1969). Because GSS is detectable in the coelomic fluid of starfish only when they are undergoing natural spawning, and not in the coelomic fluid of animals not discharging gametes, GSS was concluded to be a hormone (Kanatani and Ohguri, 1966; Kanatani and Shirai, 1970). GSS is the primary mediator of oocyte maturation in starfish, but the effect on oocyte maturation is indirect. This hormone acts on the ovary to produce a second mediator, 1-methyladenine (1-MeAde) which is the maturation-inducing hormone (MIH) of starfish (Kanatani et al., 1969; Kanatani, 1985). 1-MeAde is produced by ovarian follicle cells around oocytes upon stimulation with GSS (Hirai and Kanatani, 1971; Hirai et al., 1973), and this action of GSS is mediated through the activation of its receptor, G-protein and adenylyl cyclase (Mita et al., 1987, 1989; Mita and Nagahama, 1991). Subsequently, 1-MeAde acts on the oocyte surface to activate a third mediator M-phase- or maturation-promoting factor (MPF) (Kishimoto and Kanatani, 1976; Kishimoto et al., 1984). MPF was identified as a complex of cyclin B and Cdk1 with Greatwall (Gautier et al., 1988, 1990; Okumura et al., 2014; Hiraoka et al., 2016).

When the process of oocyte maturation in starfish is compared with that of various vertebrates (Kanatani, 1979, 1985), GSS seemed to correspond to the pituitary gonadotropin of vertebrates. However, GSS is not a pituitary glycoprotein hormone but a neuroendocrine peptide hormone (Kanatani et al., 1971). Fifty years after the initial finding of Chaet and McConnaughy (1959), GSS was finally purified from the radial nerve cords of starfish *Patiria* (*Asterina*) *pectinifera* and its chemical structure identified (Mita et al., 2009). The purified hormone is a heterodimer composed of two different peptides, A- and B-chains with two inter-chain and one intra-chain disulfide bonds. Based on its cysteine motif, starfish GSS is classified as a member of the insulin/insulin-like growth factor (IGF)/relaxin superfamily and, more precisely, it belongs to a relaxin-like peptide family (Mita et al., 2009; Mita, 2013). Therefore, GSS in starfish is designated as relaxin-like gonad-

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stimulating peptide (RGP) (Mita, 2016).

Previous studies have shown that RGP is produced as a prohormone like insulin (Mita et al., 2009). The signal peptide is followed by the B-chain, and the A-chain is located at the C-terminus. There is an intermediate sequence (C-peptide) between the B- and A-chains, which have typical proteolytic cleavage sites, Lys and Arg, at the ends. Thus, after the formation of three disulfide cross-linkages between the A- and B-chains and within the A-chain, mature RGP is produced by elimination of the signal and C-peptides.

Furthermore, cross-experiments using different species of starfish have shown that GSS generally acts non-species specifically, with some exceptions (Noumura and Kanatani, 1962; Chaet, 1966a,b). For example, radial nerve extracts (crude GSS) of *P. pectinifera* induce oocyte maturation and ovulation in the ovaries of *Asterias amurensis.* In contrast, GSS of *A. amurensis* is inactive in *P. pectinifera* ovaries. This suggested that the chemical structure of *A. amurensis* RGP (AamRGP) was different from that of *P. pectinifera* RGP (PpeRGP). Recently, the chemical structures of RGPs were identified in several species of starfish (Ikeda et al., 2015; Mita et al., 2015a,b; Lin et al., 2016; Mita and Katayama, 2016; Smith et al., 2017). There were three kinds of RGP orthologs, PpeRGP, AamRGP, and *Aphelasterias japonica* RGP (AjaRGP), among the class Asteroidea (Mita, 2016). Although the amino acid sequences of AamRGP and AjaRGP are similar to each other, their amino acid identity levels are quite different from that of PpeRGP (Mita et al., 2015a; Mita and Katayama, 2016). Neither AamRGP nor AjaRGP induces spawning in ovarian fragments of *P. pectinifera* (Mita et al., 2015a; Mita and Katayama, 2016). In contrast, PpeRGP is active in *A. amurensis* and *A. japonica* ovaries (Mita and Katayama, 2016). Thus, it is considered that partial species-specificity observed in AamRGP and AjaRGP is caused by interaction with the receptor. Because RGP is a heterodimeric peptide, either the A- or B-chain of RGP should be able to bind with its receptor.

In this study, chimeric RGP derivatives with exchanged A- and B-chains were synthesized chemically, and we examined the effect on spawning activity. The ovaries of *P. pectinifera* were used for spawning assays, because AamRGP and AjaRGP are inactive in *P. pectinifera* ovaries (Mita et al., 2015a, Mita and Katayama, 2016).

2. Materials and methods

2.1. Animals

Starfish, *P. pectinifera*, were collected from Yokosuka (Kanagawa, Japan), Choshi (Chiba, Japan), Ushimado (Okayama, Japan), and Asamushi (Aomori, Japan). Animals were kept in circulating artificial seawater at 15 °C and used within 2 months after collection.

2.2. Peptide synthesis

RGP and chimeric derivatives were synthesized essentially in accordance with the method for synthesizing insulin-like peptides as described previously (Katayama et al., 2014) (Katayama and Mita, 2016). In brief, the A- and B-chains were prepared by the ordinary 9-fluorenylmethoxycarbonyl (Fmoc)-based solid-phase peptide synthesis. Three disulfide bonds were regioselectively formed by dimethyl sulfoxide (DMSO) oxidation, *S*-pyridylsulfenyl-directed thiolysis, and iodine oxidation reactions. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) was performed using an Autoflex spectrometer (Brucker). Amino acid composition was determined using a LaChrom amino acid analyzer (Hitachi, Tokyo, Japan) after hydrolysis with a 6 M HCl solution at 150 °C for 2 h in a vacuum-sealed tube. Further chemical synthesis methods and analytical data for each synthetic peptide consisting of RGP A- and B-chain (RGP-A/RGP-B) were described in supporting information.

2.3. Induction of oocyte maturation and ovulation

The bioactivities of the synthetic RGPs were assayed using ovarian fragments from *P. pectinifera* as described previously (Shirai, 1986). Modified van't Hoff's artificial seawater (ASW) adjusted to pH 8.2 with 0.02 M borate buffer was prepared (Kanatani and Shirai, 1970), and the ovaries of mature female starfish were excised and cut using scissors into small fragments containing only a few lobes. The ovarian fragments were then incubated in ASW containing peptides at a range of concentrations (0.4–50 nM) for 1 h. The samples were examined to determine whether or not spawning had occurred and were scored (Shirai, 1986) as follows: (+ + +) spawning occurred and most oocytes had matured; (+ +) about 50% of oocytes had matured, (+) a few oocytes had matured, and (−): no spawning occurred. The scores were converted to numerical values (+ + + = 100; + + = 67; + = 33; − = 0) so that the effective dose for inducing gamete spawning in 50% of ovarian fragments (EC_{50}) could be determined graphically. Means ± SEM were determined from four separate assays using ovaries from different animals.

2.4. Circular dichroism spectral analysis

Circular dichroism (CD) spectra of the synthetic RGPs including chimeric derivatives were measured with a Jasco J-820 spectropolarimeter (JASCO, Tokyo, Japan) at room temperature with a 2-mm path length cell using phosphate buffer (50 mM, pH 7.0) as a solvent. The α-helical content (f_H) of each synthetic peptide was calculated by molar ellipticity ([θ]) at 222 nm using the following formula: $f_H = -([\theta]_{222} + 2340)/30300$ (Chen and Yang, 1971; Chen et al., 1972).

2.5. Three-dimensional structure models

Three-dimensional (3D) structure models of synthetic PGPs including chimeric derivatives were predicted using SWISS-MODEL (https://swissmodel.expasy.org/, Guex et al., 2009; Benkert et al., 2011) with default setting. The structural template consisting of sequences combined with the signal peptide to B-chain and the C-peptide to A-chain among three kinds of pre-proRGPs (Fig. 1A) was automatically set by the software to the solution structure of human insulin or IGF (PDB code, 2GF1). The modeled structures were visualized with Pymol (http://www.pymol.org), then the signal and C-peptides were eliminated.

3. Results

Because hormonal action is transmitted through receptors, the partial species-specificity observed in some RGPs should be caused by the interaction with the receptor. Previous studies have shown that three kinds of RGP orthologs, PpeRGP, AamRGP, and AjaRGP, are identified in this class Asteroidea (Mita, 2016). To examine the interaction between RGP and its receptor, nine kinds of peptides including chimeric RGP derivatives with replaced A- (RGP-A) and B-chain (RGP-B) from these RGPs were synthesized chemically (Fig. 1B).

When ovarian fragments from *P. pectinifera* were incubated in ASW containing these peptides, spawning was observed with PpeRGP, PpeRGP-A/AamRGP-B, and PpeRGP-A/AjaRGP-B after 60 min of incubation. The spawning activity was retained in chimeric PpeRGP derivatives, replaced the B-chain with that of AamRGP or AjaRGP. EC_{50} values were obtained of approximately 1.1 nM for PpeRGP, 13.9 nM for PpeRGP-A/AamRGP-B, and 9.8 nM for PpeRGP-A/AjaRGP-B (Table 1). In contrast, spawning did not occur in RGP derivatives containing the A-chain of AamRGP or AjaRGP (Table 1).

To understand molecular character of peptides in detail, CD spectra from PpeRGP, AamRGP, AjaRGP, and their chimeric derivatives were examined (Fig. 2). Compared with the CD spectra of peptides involving

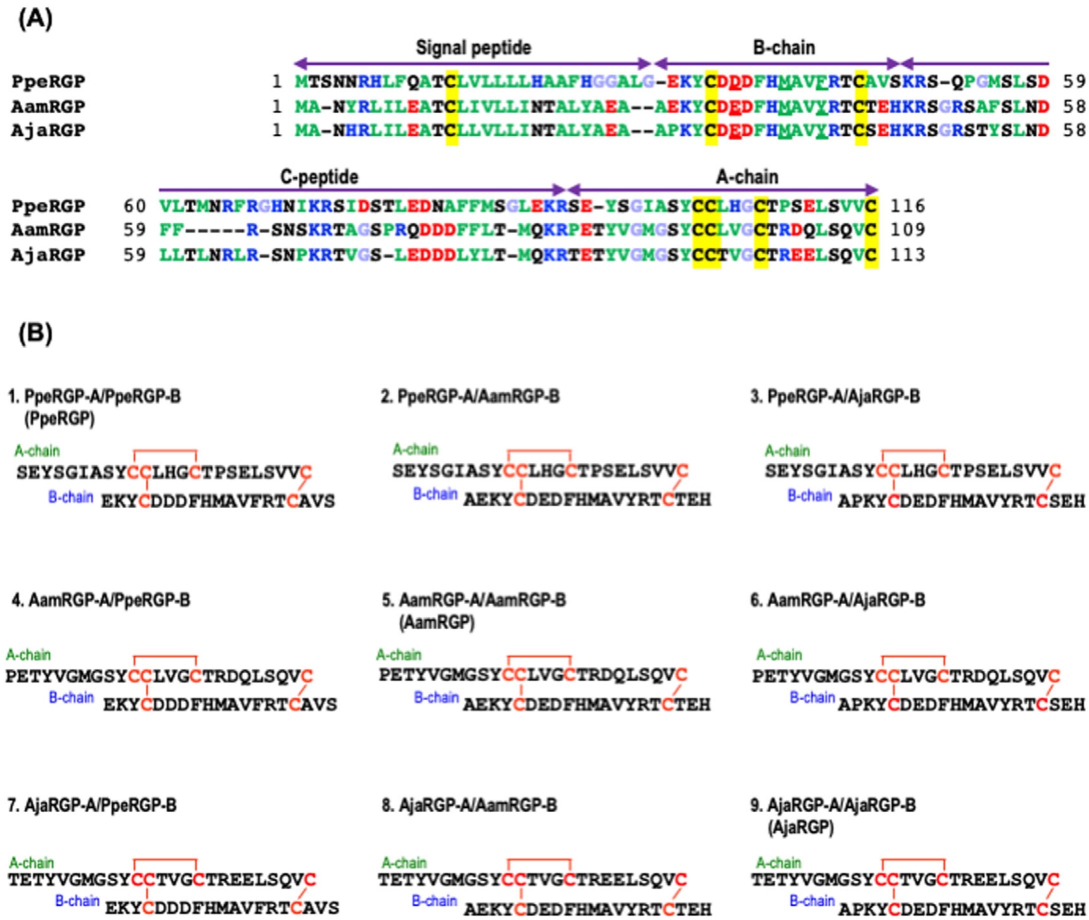

Fig. 1. Amino acid sequences of relaxin-like gonad-stimulating peptides (RGPs) and their chimeric derivatives. (A) Alignment of pre-prohormone sequences of RGP in the starfish *Patiria pectinifera* (PpeRGP), *Asterias amurensis*, (AamRGP), and *Aphelasterias japonica* (AjaRGP). The signal peptide, B-chain, C-peptide, and A-chain are indicated. To illustrate the conserved features, the amino acid types are color coded according to their nature with basic residues in blue (Arg, Lys and His), acidic residues in red (Glu and Asp), hydrophobic residues in green (Ala, Val, Ile, Phe, Trp, Tyr, Pro and Met), hydrophilic in black (Ser, Thr, Asn and Gln) and glycine in light blue. The cysteine residues are highlighted in yellow. The key residues for receptor binding are underlined. (B) Chemical structures of PpeRGP, AamRGP, AjaRGP, and their chimeric derivatives. Characters shown in red indicate cysteine residues. The cysteine bridges are shown as red, solid lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Effects of chimeric RGP derivatives on the induction of oocyte maturation and ovulation in ovarian fragments of *Patiria pectinifera.*

Peptides (A-chain/B-chain)	EC_{50} (nM)
1. PpePGP-A/PpeRGP-B (PpeRGP)	1.1 ± 0.2
2. PpeRGP-A/AamRGP-B	13.9 ± 2.6
3. PpeRGP-A/AjaRGP-B	9.8 ± 1.7
4. AamRGP-A/PpeRGP-B	Inactive*
5. AamRGP-A/AamRGP-B (AamRGP)	Inactive*
6. AamRGP-A/AjaRGP-B	Inactive*
7. AjaRGP-A/PpeRGP-B	Inactive*
8. AjaRGP-A/AamRGP-B	Inactive*
9. AjaRGP-A/AjaRGP-B (AjaRGP)	Inactive*

Ovarian fragments were incubated with various concentrations of chimeric RGP derivatives for 1 h. The effective dose for induction of oocyte spawning in 50% of ovarian fragments (EC_{50}) was determined from four experiments. Values are means ± SEM of four separate assays using different animals.
*Peptides used were examined up to 50 nM.

the PpeRGP A-chain, images of [θ] in PpeRGP, PpeRGP-A/AamRGP-B, and PpeRGP-A/AjaRGP-B were almost the same between 190 and 260 nm. Contents of α-helix in PpeRGP, PpeRGP-A/AamRGP-B, and PpeRGP-A/AjaRGP-B were estimated between 30.0 and 39.1% (Fig. 2). In contrast, the CD spectra, particularly around 195 nm, of chimeric RGP derivatives such as AamRGP-A/PpeRGP-B, AjaRGP-A/PpeRGP-B, and AjaRGP-A/AamRGP-B were quite different from those of AamRGP and AjaRGP. The α-helical contents in AamRGP-A/PpeRGP-B, AjaRGP-A/PpeRGP-B, and AjaRGP-A/AamRGP-B were less than 25%. Although AamRGP, AjaRGP, and AamRGP-A/AjaRGP-B could not induce spawning in *P. pectinifera* ovaries (Table 1), their CD spectra were mostly similar to that of PpeRGP (Fig. 2).

Because the interaction with the receptor is related to the 3D structure of the ligand, 3D structure models of the RGPs and their chimeric derivatives were built using SWISS-MODEL https://swissmodel.expasy.org/ (Guex et al., 2009; Benkert et al., 2011). The predicted 3D structure models showed three disulfide cross-linkages; two interchain between the A- and B-chains, and an intrachain within the A-chain (Fig. 3A). It is considered that the 3D models are close to the native structures. The 3D structures of the B-chains in PpeRGP, AamRGP, AjaRGP and their chimeric derivatives showed almost the same conformation (Fig. 3A). This suggests that the structure of the B-chain is almost consistent with each other. In contrast, the 3D structures of the A-chains seemed to be quite different among RGP derivatives. Therefore, it is possible that the B-chain of RGP is involved in binding to the receptor.

4. Discussion

Oocyte maturation in starfish is induced by 1-MeAde produced in

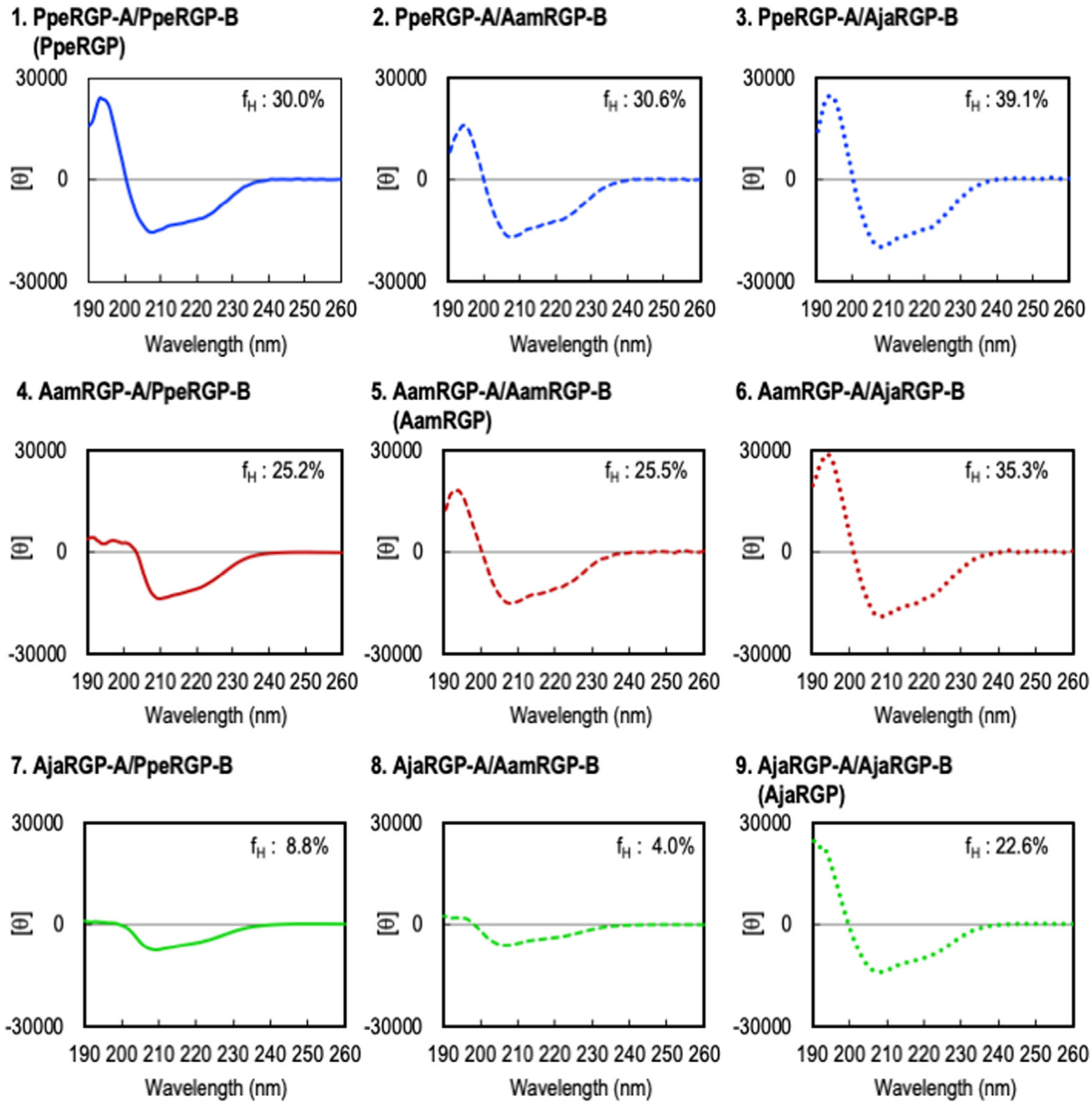

Fig. 2. Circular dichroism (CD) spectra of relaxin-like gonad-stimulating peptides (RGPs) and their chimeric derivatives. The spectra are derived from nine kinds of RGP derivatives with exchanged each A- and B-chain of *P. pectinifera* (PpeRGP), *A. amurensis*, (AamRGP), and *A. japonica* (AjaRGP). Lines shown in blue, red, and green indicate the spectra of peptides composed of the PpeRGP A-chain (-A), AamRGP-A, and AjaRGP-A, respectively. Solid, dashed, and dotted lines show the peptide spectra composed of the PpeRGP B-chain (-B), AamRGP-B, and AjaRGP-B. The α-herical content (f_H) of RGP derivatives was calculated as follows: $f_H = -([\theta]_{222} + 2340)/30300$ (Chen and Yang, 1971; Chen et al., 1972). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ovarian follicle cells under the influence of RGP released from the nervous tissue. Though 1-MeAde is a common MIH in the class Asteroidea (Kanatani, 1985), RGP acts partially species-specifically (Noumura and Kanatani, 1962; Chaet, 1966a,b). Previous studies have reported that AamRGP and AjaRGP fail to induce oocyte maturation and ovulation in *P. pectinifera* ovaries (Mita et al., 2015a; Mita and Katayama, 2016). In this study, similar to PpeRGP, its chimeric derivatives with B-chain from AamRGP or AjaRGP could induce spawning (Table 1). In contrast, spawning activity was not observed in peptides involving the AamRGP A-chain and AjaRGP A-chain. These results may suggest that the A-chain of PpeRGP is important for inducing spawning in *P. pectinifera* ovaries.

This study showed that the CD spectra of PpeRGP-A/AamRGP-B and PpeRGP-A/AjaRGP-B were similar to that of PpeRGP (Fig. 2). In contrast, the CD spectra of AamRGP and AjaRGP changed drastically when their B-chains were replaced by the PpeRGP B-chain. The α-helical contents of PpeRGP, PpeRGP-A/AamRGP-B, and PpeRGP-A/AjaRGP-B were relatively higher than those of AamRGP-A/PpeRGP-B and AjaRGP-A/PpeRGP-B. In this study, spawning activity was observed in PpeRGP derivatives with high α-helical contents. This strongly suggests that a conformation of RGP molecule is important for binding to the receptor.

The predicted 3D structure models of the A-chain seemed to be quite different among RGP derivatives (Fig. 3A). In contrast, the 3D structure models of the B-chains seemed to have the same conformation. Probably, replacing the B-chain of PpeRGP with another RGP type has little effect on binding to the receptor. It is thus considered that PpeRGP, its chimeric derivatives with B-chain from AamRGP or AjaRGP could induce spawning in P. *pectinifera* ovaries. Previous studies have also showed that PpeRGP can induce spawning in ovaries of *A. amurensis* (Mita et al., 2015a) and *A. japonica* (Mita and Katayama, 2016). This suggests that the B-chain of RGP is more important than the A-chain for binding to the receptor.

In this study, although the CD spectra of AamRGP, AjaRGP, and AamRGP-A/AjaRGP-B were mostly similar to that of PpeRGP (Fig. 2), those RGP derivatives failed to induce spawning in *P. pectinifera* ovaries

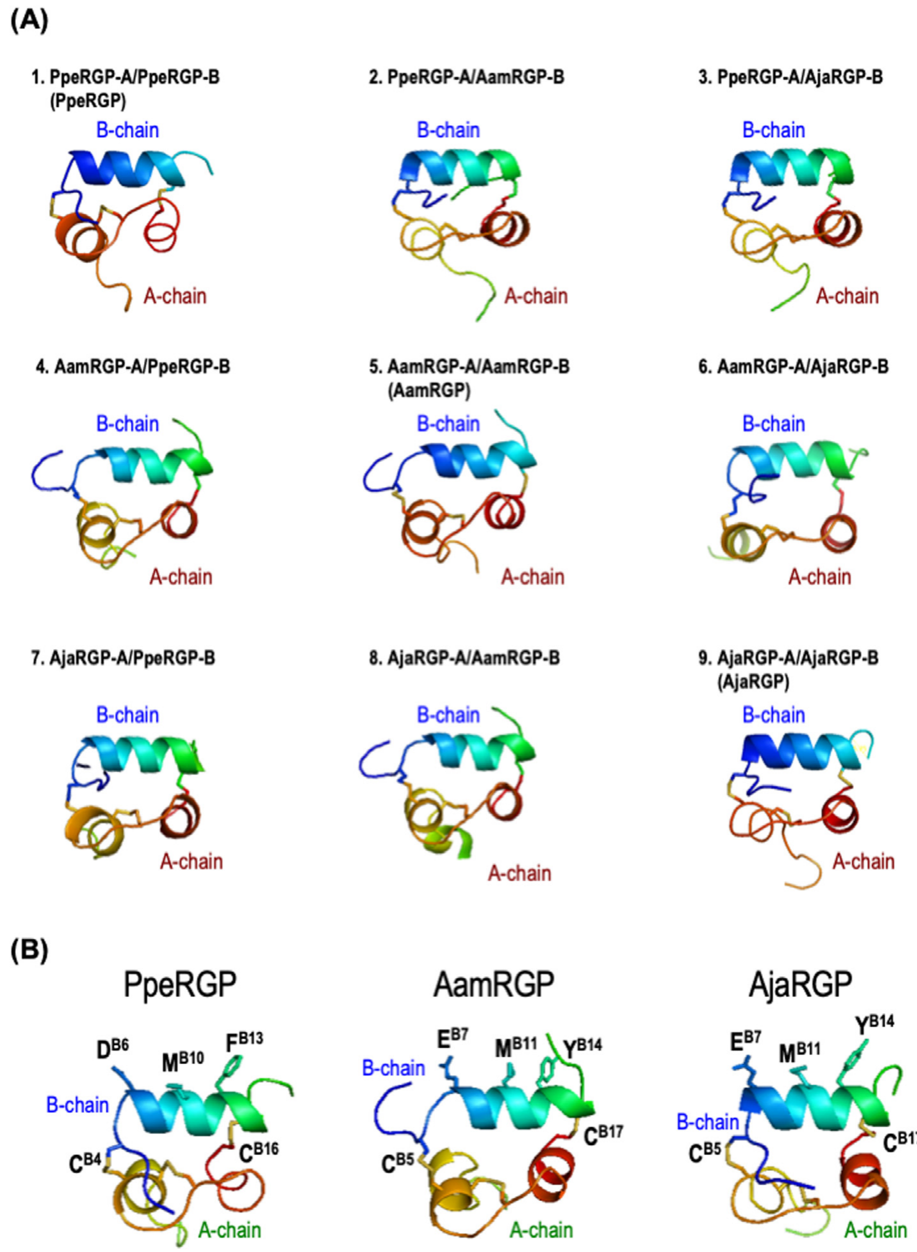

Fig. 3. Three-dimensional (3D) structure models of relaxin-like gonad-stimulating peptides (RGPs) and their chimeric derivatives. (A) 3D structure models of nine kinds of RGP derivatives with exchanged each A- and B-chain of *P. pectinifera* (PpeRGP), *A. amurensis*, (AamRGP), and *A. japonica* (AjaRGP). Each 3D structure model was built using SWISS-MODEL as described in Section 2. (B) Predicted amino acid residues of PpeRGP, AamRGP and AjaRGP to interact with a receptor protein.

(Table 1). It is possible that the A-chain of AamRGP and AjaRGP disturbs the binding between the B-chain of PpeRGP and the receptor. Otherwise, even though a peptide binds to its receptor, the receptor may not activate the process of signal transduction.

In the breeding season, RGP secreted from the radial nerve cords reaches the ovary through the sinus system (Kanatani, 1979, 1985) and binds to the receptor on the surface of ovarian follicle cells. The results obtained in this study support that the B-chain of RGP plays an important role in the interaction with the receptor. Previous studies have shown that receptors for the relaxin family peptides (RXFPs) belong to the superfamily of rhodopsin-like G-protein-coupled receptors (GPCRs) (Bathgate et al., 2006; Alexander et al., 2015). In humans, the relaxin superfamily consists of relaxin 1 (H1 relaxin), relaxin 2 (H2 relaxin), relaxin 3 (H3 relaxin), insulin-like peptide 3 (INSL3 also known as relaxin-like factor or Leydig insulin-like peptide), insulin-like peptide 4 (INSL4 or placetin), insulin-like peptide 5 (INSL5), and insulin-like peptide 6 (INSL6) (Hudson et al., 1983; Crawford et al., 1984; Adham et al., 1993; Chassin et al., 1995; Conklin et al., 1999; Chang et al., 2000; Bathgate et al., 2002; Alexander et al.,2015). H2 relaxin, INSL3, H3 relaxin, and INSL5 signal through RXFP1 (Hsu et al., 2002), RXFP2 (Kumagai et al., 2002), RXFP3 (Liu et al., 2003) and RXFP4 (Liu et al., 2005) receptors, respectively. Although it is considered that H1 relaxin is a ligand of the RXFP1 (Bathgate et al., 2013; Patil et al., 2017, Bathgate et al., 2018), the native receptors for INSL4 and INSL6 are yet to be identified (Halls et al., 2007). All the family members of relaxin peptides and their targets receptors have broad physiological roles (Bathgate et al., 2013; Patil et al., 2017; Bathgate et al., 2018). The activation of adenylyl cyclase by RXFP1 is complex and involves the interaction with several G-proteins, resulting in a biphasic pattern of cAMP accumulation (Bathgate et al., 2013). RXFP2 activates adenylyl cyclase *in vitro* but some physiological responses are sensitive to pertussis toxin (Bathgate et al., 2013). The signaling pathways activated by

RXFP3 or RXFP4 result in the inhibition of adenylyl cyclase and a decrease in cAMP accumulation. This suggests that RGP receptor belongs to RXFP1/RXFP2 rather than RXFP3/RXFP4, although the receptor has not been identified yet.

Previous studies have shown in vertebrates that a 'relaxin-specific receptor-binding cassette' (Arg XXX Arg XX Ile/Val) in the B-chain of relaxin is important to bind to the receptor (Büllesbach and Schwabe, 2000). Despite its similarity with relaxins, however, the RGP sequence does not possess the vertebrate 'relaxin-specific receptor-binding cassette' in the B-chain (Fig. 1A). A comparison of amino acid sequences of middle region of B-chains indicates that residues of the 'receptor-binding cassette' correspond to Asp^{B6}, Met^{B10} and F^{B13} for PpeRGP and Glu^{B7}, Met^{B11}, and Tyr^{B14} for AamRGP and AjaRGP, respectively (Fig. 1A). Observing with 3D structure models, the key residues are predicted to be located helical turn away in B-chains of PpeRGP, AamRGP, and AjaRGP (Fig. 3B). Although it is an excessive speculation, it may be possible that the key residues in B-chain of RGP are important for binding to the receptor. Starfish RGP and vertebrate relaxin are probably derived from the same ancestral peptide. Further studies on the receptor protein for RGP could provide useful insights into the hormonal action and evolution of species differentiation in the class Asteroidea.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2019.02.019.

References

Adham, I.M., Burkhardt, E., Benahmed, M., Engel, W., 1993. Cloning of a cDNA for a novel insulin-like peptide of the testicular Leydig cells. J. Biol. Chem. 268, 26668–26672.

Alexander, S.P.H., Davenport, A.P., Kelly, E., Marrio, N., Peters, J.A., Benson, H.E., Faccenda, E., Pawson, A.J., Sharman, J.L., Southan, C., Davis, J.A., et al., 2015. The concise guide to PHARMACOLOGY 2015/16: G protein-coupled receptors. Br. J. Pharmacol. 172, 5744–5861.

Bathgate, R.A.D., Samuel, C.S., Burazin, T.C.D., Layfield, S., Claasz, A.A., Reytomas, I.G.T., Dawson, N.F., Zhao, C., Bond, C., Summers, R.J., Parry, L.J., Wade, J.D., Tregear, G.W., 2002. Human relaxin gene 3 (*H3*) and the equivalent mouse relaxin (*M3*) gene. J. Biol. Chem. 277, 1148–1157.

Bathgate, R.A., Ivell, R., Sanborn, B.M., Sherwood, O.D., Summers, R.J., 2006. International Union of Pharmacology LVII: recommendations for the nomenclature of receptors for relaxin family peptides. Pharmacol. Rev. 58, 7–31.

Bathgate, R.A.D., Halls, M.L., van der Westhuizen, E.T., Callander, G.E., Kocan, M., Summers, R.J., 2013. Relaxin family peptides and their receptors. Physiol. Rev. 93, 405–480.

Bathgate, R.A.D., Kocan, M., Scott, J.D., Hossain, M.A., Good, S.V., Yegorov, S., Bogerd, J., Gooley, P.R., 2018. Relaxin family peptides and their receptors. Pharmacol. Ther. 187, 114–132.

Benkert, P., Biasini, M., Schwede, T., 2011. Toward the estimation of the absolute quality of individual protein structure models. Bioinformatics 27, 343–350.

Büllesbach, E.E., Schwabe, C., 2000. The relaxin receptor-binding site geometry suggests a novel gripping mode of interaction. J. Biol. Chem. 275, 35276–35280.

Chaet, A.B., McConnaughy, R.A., 1959. Physiologic activity of nerve extracts. Biol. Bull. 117, 407–408.

Chaet, A., 1966a. The gamete-shedding substances of starfishes: a physiological-biochemical study. Am. Zoologist 6, 263–271.

Chaet, A., 1966b. Neurochemical control of gamete release in starfish. Biol. Bull. 130, 43–58.

Chang, G.T., Steenbeek, M., Schippers, E., Blok, L.J., van Weeerden, W.M., Alewijk, D.C., Eussen, B.H., van Steenbrugge, G.L., Brinkmann, A.O., 2000. Characterization of a zinc-finger protein and its association with apoptosis in prostate cancer cells. J. Natl. Cancer Inst. 92, 1414–1421.

Chassin, D., Laurent, A., Janneau, J.L., Berger, R., Bellet, D., 1995. Cloning of a new member of the insulin gene superfamily (INSL4) expressed in human placenta. Genomics 29, 1414–1421.

Chen, Y.H., Yang, J.T., 1971. A new approach to the calculation of secondary structures of globular proteins by optical rotatory dispersion and circular dichroism. Biochem. Biophys. Res. Commun. 44, 1285–1291.

Chen, Y.H., Yang, J.T., Martinez, H.M., 1972. Determination of the secondary structures of proteins by circular dichroism and optical rotatory dispersion. Biochemistry 11, 4120–4131.

Conklin, D., Lofton-Day, C.E., Haldeman, B.A., Ching, A., Whitmore, T.E., Lok, S., Jaspers, S., 1999. Identification of INSL5, a new member of the insulin superfamily. Genomics 60, 50–56.

Crawford, R.J., Hudson, P., Shine, J., Niall, H.D., Eddy, R.L., Shows, T.B., 1984. Two human relaxin genes are on chromosome 9. EMBO J. 3, 2341–2345.

Gautier, J., Norbury, C., Lohka, M., Nurse, P., Maller, J., 1988. Purified maturation-promoting factor contains the product of a *Xenopus* homolog of fission yeast cell cycle control gene *cdc2*$^+$. Cell 54, 433–439.

Gautier, J., Minshull, J., Lohka, M., Glotzer, M., Hunt, T., Maller, J.L., 1990. Cyclin is a component of maturation-promoting factor from *Xenopus*. Cell 60, 487–494.

Guex, N., Peitsch, M.C., Schwede, T., 2009. Automated comparative protein structure modeling with SWISS-MODEL and Swiss-PdbViewer: a historical perspective. Electrophoresis 30, S162–S173.

Halls, M.L., van der Westhuizen, E.T., Bathgate, R.E., Summers, R.J., 2007. Relaxin family peptide receptors-former orphans reunite with their parent ligands to activate multiple signaling pathways. Br. J. Pharmacol. 150, 677–691.

Hirai, S., Kanatani, H., 1971. Site of production of meiosis-inducing substance in ovary of starfish. Exp. Cell Res. 57, 224–227.

Hirai, S., Chida, K., Kanatani, H., 1973. Role of follicle cells in maturation of starfish oocytes. Dev. Growth Differ. 15, 21–31.

Hiraoka, D., Aono, R., Hanada, S., Okumura, E., Kishimoto, T., 2016. Two new competing pathways establish the threshold for cyclin B-Cdk1 activation at the meiotic G2/M transition. J. Cell Sci. 129, 3153–3166.

Hsu, S.Y., Nakabayashi, K., Nishi, S., Kumagai, J., Kudo, M., Sherwood, O.D., Hsueh, A.J.W., 2002. Activation of orphan receptors by the hormone relaxin. Science 295, 671–674.

Hudson, P., Haley, J., John, M., Cronk, M., Crawford, R., Haralambidis, J., Tregear, G., Shine, J., Niall, H., 1983. Structure of a genomic clone encoding biologically active human relaxin. Nature 301, 628–631.

Ikeda, N., Uzawa, H., Daiya, M., Haraguchi, S., Tsutsui, K., Mita, M., 2015. Relaxin-like gonad-stimulating peptide is a highly conserved peptide in starfish *Asterina pectinifera*. Invert. Reprod. Dev. 59, 224–229.

Kanatani, H., Ohguri, M., 1966. Mechanism of starfish spawning. I. Distribution of active substance responsible for maturation of oocytes and shedding of gametes. Biol. Bull. 131, 104–114.

Kanatani, H., Shirai, H., 1967. *In vitro* production of meiosis-inducing substance by nerve extract in ovary of starfish. Nature 216, 284–286.

Kanatani, H., Shirai, H., 1969. Mechanism of starfish spawning. II. Some aspects of action of a neural substance obtained from radial nerve. Biol. Bull. 137, 297–311.

Kanatani, H., Shirai, H., Nakanishi, K., Kurokawa, T., 1969. Isolation and identification of meiosis-inducing substance in starfish, *Asterias amurensis*. Nature 221, 273–274.

Kanatani, H., Shirai, H., 1970. Mechanism of starfish spawning. III. Properties and action of meiosis-inducing substance produced in gonad under influence of gonad-stimulating substance. Dev. Growth Differ. 12, 119–140.

Kanatani, H., Ikegami, S., Shirai, H., Oide, H., Tamura, T., 1971. Purification of gonad-stimulating substance obtained from radial nerves of the starfish *Asterias amurensis*. Dev. Growth Differ. 13, 151–164.

Kanatani, H., 1979. Hormones in echinoderms. In: Barrington, E. (Ed.), Hormones and Evolution. Academic press, London, pp. 273–307.

Kanatani, H., 1985. Oocyte growth and maturation in starfish. In: In: Metz, C.B., Monroy, A. (Eds.), Biology of Fertilization, vol. 1. Academic Press, New York, pp. 119–140.

Katayama, H., Kubota, N., Hojo, H., Okada, A., Kotaka, S., Tsutsui, N., Ohira, T., 2014. Direct evidence for the function of crustacean insulin-like androgenic gland factor (IAG): total chemical synthesis of IAG. Bioorg. Med. Chem. 22, 5783–5789.

Katayama, H., Mita, M., 2016. A sulfanyl-PEG derivative of relaxin-like peptide utilizable for the conjugation with KLH and the antibody production. Bioorg. Med. Chem. 24, 3596–3602.

Kishimoto, T., Kanatani, H., 1976. Cytoplasmic factor responsible for germinal vesicle breakdown and meiotic maturation in starfish oocyte. Nature 260, 321–322.

Kishimoto, T., Usui, N., Kanatani, H., 1984. Breakdown of starfish ovarian follicle induced by maturation-promoting factor. Dev. Biol. 101, 28–34.

Kumagai, J., Hsu, S.Y., Matsumi, H., Roh, J.S., Fu, P., Wade, J.D., Bathgate, R.A.D., Hsueh, A.J., 2002. INSL3/Leydig insulin-like peptide activates the LGR8 receptor important in testis descent. J. Biol. Chem. 277, 31283–31286.

Lin, M., Mita, M., Egertová, M., Zampronio, C.G., Jones, A.M., Elphick, M.R., 2016. Cellular localization of relaxin-like gonad-stimulating peptide expression in *Asterias rubens*: new insights into hormonal control of spawning in starfish. J. Comp. Neurol. 525, 1599–1617.

Liu, C., Chen, J., Sutton, S., Roland, B., Kuei, C., Farmer, N., Sillard, R., Lovenberg, T.W., 2003. dentification of relaxin-3/INSL7 as a ligand for GPCR142. J. Biol. Chem. 278, 50765–50770.

Liu, C., Kuei, C., Sutton, S., Chen, J., Bonaventure, P., Wu, J., Nepomuceno, D., Kamme, F., Tran, D.-T., Zhu, J., Wilkinson, T., Bathgate, R., Eriste, E., Sillard, R., Lovenberg, T.W., 2005. INSL5 is a high affinity specific agonist for GPCR142 (GPR100). J. Biol.

Chem. 280, 292–300.
Mita, M., Ueta, N., Nagahama, Y., 1987. Regulatory functions of cyclic adenosine 3',5'-monophosphate in 1-methyladenine production by starfish follicle cells. Biochem. Biophys. Res. Commun. 147, 8–12.
Mita, M., Ueta, N., Nagahama, Y., 1989. Mediation of cyclic adenosine 3',5'-monophosphate in 1-methyladenine production by starfish ovarian follicle cells. Gen. Comp. Endocrinol. 76, 241–249.
Mita, M., Nagahama, Y., 1991. Involvement of G-proteins and adenylate cyclase in the action of gonad-stimulating substance on starfish ovarian follicle cells. Dev. Biol. 144, 262–268.
Mita, M., Yoshikuni, M., Ohno, K., Shibata, Y., Paul-Prasanth, B., Pitchayawasin, S., Isobe, M., Nagahama, Y., 2009. A relaxin-like peptide purified from radial nerves induces oocyte maturation and ovulation in the starfish, *Asterina pectinifera*. Proc. Natl. Acad. Sci. U.S.A. 106, 9507–9512.
Mita, M., 2013. Relaxin-like gonad-stimulating substance in an echinoderm, the starfish: a novel relaxin system in reproduction of invertebrates. Gen. Comp. Endocinol. 181, 241–245.
Mita, M., Daiya, M., Haraguchi, S., Tsutsui, K., Nagahama, Y., 2015a. A new relaxin-like gonad-stimulating peptide identified in the starfish *Asterias amurensis*. Gen. Comp. Endocinol. 222, 144–149.
Mita, M., Ikeda, N., Haraguchi, S., Tsutsui, K., Nakamura, M., 2015b. A gonad-stimulating peptide of the crown-of-thorns starfish, *Acanthaster planci*. Invert. Reprod. Dev. 59, 212–217.
Mita, M., 2016. Starfish gonadotropic hormone: relaxin-like gonad-stimulating peptides. Gen. Comp. Endocinol. 230–231, 166–169.
Mita, M., Katayama, H., 2016. A relaxin-like gonad-stimulating peptide from the starfish *Aphelasterias japonica*. Gen. Comp. Endocrinol. 229, 56–61.
Noumura, T., Kanatani, H., 1962. Induction of spawning by radial nerve extracts in some starfishes. J. Fac. Sci. Univ. Tokyo Sect. IV 9, 397–402.
Okumura, E., Morita, A., Wakai, M., Mochida, S., Hara, M., Kishimoto, T., 2014. Cyclin B-Cdk1 inhibits protein phosphatase PP2A-B55 via a Greatwall kinase-independent mechanism. J. Cell Biol. 204, 881–889.
Patil, N.A., Rosengren, K.J., Separovic, F., Wade, J.D., Bathgate, R.A.D., Hossain, M.A., 2017. Relaxin family peptides: structure-activity relationship studies. Br. J. Pharmacol. 174, 950–961.
Shirai, H., 1986. Gonad-stimulating and maturation-inducing substance. In: In: Schroeder, T.E. (Ed.), Methods in Cell Biology, vol. 27. Academic Press, Orlando, pp. 73–83.
Smith, M.K., Wang, T., Suwansa-ard, S., Motti, C.A., Elizur, A., Zhao, M., Rowe, M.L., Hall, M.R., Elphick, M.R., Cummins, S.F., 2017. The neuropeptidome of the Crown-of-Thorns Starfish *Acanthaster planci*. J. Proteom. 165, 61–68.