Published in final edited form as:

Bioorg Med Chem Lett. 2010 October 15; 20(20): 6040–6044. doi:10.1016/j.bmcl.2010.08.062.

Elucidation of the active conformation of the amino terminus of receptor-bound secretin using intramolecular disulfide bond constraints

Maoqing Dong, Delia I. Pinon, Andrew J. Bordner, and Laurence J. Miller*

Department of Molecular Pharmacology and Experimental Therapeutics, Mayo Clinic, Scottsdale, AZ 85259

Abstract

Family B G protein-coupled receptors include several potentially important drug targets, yet our understanding of the molecular basis of ligand binding to and activation of these receptors is incomplete. While NMR and crystal structures exist for peptide ligand-associated amino-terminal domains of several family members, these only provide insights into the conformation of the carboxyl-terminal region of the peptides. The amino-terminal region of these peptides, critical for biological activity, is believed to interact with the helical bundle domain, and is, therefore, unconstrained in these structures. The aim of the current study was to provide insights into the conformation of the amino terminus of secretin as bound to its receptor. We prepared a series of conformationally-constrained secretin peptides containing intramolecular disulfide bonds that were predicted by molecular modeling to approximate the conformation of the analogous region of PACAP bound to its receptor that had been determined using transfer-NOE NMR techniques. Secretin peptides with pairs of cysteine residues in positions 2–7, 3–5, 3–6, 4–7, 7–9, and 4–10 were studied as linear and disulfide-bonded forms. The analogue with a disulfide bond connecting positions 7–9 had binding affinity and biological activity similar to natural secretin, supporting the relevance of this constraint to its active conformation. While this feature is shared between secretin and PACAP, absence of activity in other constrained peptides in this series also suggest that there are differences between these receptor-bound conformations. It will be critical to extend similar studies to other family members to learn what structural elements might be most conserved in this family.

Keywords

secretin; secretin receptor; family B G protein-coupled receptor; active peptide conformation; ligand binding

By first principles, linear peptides tend to have many degrees of conformational freedom. Their inherent flexibility provides a substantial challenge when attempting to elucidate a meaningful conformation of a peptide ligand docked at its receptor. Crystal structures and NMR structures that contain both peptide ligands and receptor molecules can provide useful

© 2010 Elsevier Ltd. All rights reserved

*Please send all correspondence and reprint requests to: Laurence J. Miller, M.D. Mayo Clinic 13400 East Shea Boulevard Scottsdale, AZ 85259 Telephone: (480) 301-4217 Fax: (480) 301-8387 miller@mayo.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

insights, particularly if there is evidence that the docked conformation reflects normal binding affinity and/or is capable of stimulating biological activity. Unfortunately, many such structures may be more reflective of low affinity, non-specific inter-molecular interactions, such as being driven by hydrophobic interactions between the molecules, which may have limited relevance to natural docking.

Several high-resolution NMR and crystal structures exist for natural peptide ligands or their analogues associated with refolded amino-terminal domains of family B guanine nucleotide-binding protein (G protein)-coupled receptors.^{1–6} While these structures provide relatively consistent insights into the docking of the carboxyl-terminal regions of the peptide ligands that typically assume a helical conformation and reside within a binding cleft within the receptor amino terminus,^{1–6} unfortunately, none of these structures include the amino-terminal regions of their ligands. This reflects the observation that the amino terminus of the ligands normally interacts with the helical bundle of these receptors,^{7–10} and that there is nothing to constrain the structures of that portion of the ligands in these complexes. Indeed, it is the amino-terminal region of these ligands that has been demonstrated to be most responsible for their biological activity.^{11,12}

There is only a single structure reported that directly provides insights into the amino-terminal region of a peptide ligand in this family docked at its receptor.¹³ This study utilized transfer-NOE NMR techniques that provide information only for the ligand and not for the underlying receptor. This structure (PDB entry 1GEA) represents pituitary adenylate cyclase activating polypeptide truncated at its carboxyl terminus, PACAP(1–21), bound to the PACAP receptor. The peptide backbone in this structure assumes an extended conformation from residues 1–3, two overlapping β turns, comprised of residues 3–6 and 4–7, and a carboxyl-terminal α -helix from residues 8–21.

Based on the hypothesis that the secretin and PACAP peptides adopt similar receptor-bound conformations, we have used the structure of the amino terminus of PACAP as a template for modeling possible receptor-bound conformations of this region of the secretin peptide in the current project. We have constrained the structure of secretin analogues using intramolecular disulfide bonds and have characterized the abilities of these analogues to bind to the intact receptor and to elicit cAMP responses in receptor-bearing cells.

Molecular modeling was used to predict where a disulfide bond could be introduced into the amino-terminal portion of the secretin peptide to achieve a backbone structure similar to that found in the receptor-bound PACAP structure. All modeling was performed using the Internal Coordinate Mechanics (ICM) molecular mechanics program (Molsoft LLC, La Jolla, CA). First, a series of all-atom models was generated for the secretin peptide, incorporating two cysteines and a disulfide bond between them. All possible pairs of cysteine residues in the range of positions 2 through 11, which were separated by at least two residues in the linear sequence, were examined. Next, harmonic restraints with weight 10 kcal/(mol $\text{\AA}^2$) were defined between corresponding backbone atoms in the secretin peptide and in the PACAP structure. The secretin peptide has high sequence similarity (43% identity) to PACAP(1–21), providing a confident gapless alignment to infer corresponding residues. The total energy, which is the sum of the physical energy calculated using the ECEPP/3 force field and the harmonic restraint potential, was minimized using the optimal-bias Monte Carlo optimization procedure¹⁴ in ICM. The Metropolis sampling temperature was set at 700K and each simulation was run for a total of 10^5 energy evaluations. Simulations were run using each of the 25 deposited structures in the 1GEA PDB entry as backbone templates. The consistency of each of the predicted structures for disulfide-linked secretin was evaluated by its physical energy, disulfide bond geometry, and deviation from the bound PACAP backbone structure. A typical disulfide bond, as inferred from high-

resolution X-ray structures, has a C β -S-S-C β dihedral angle near 90° and an S-S bond length of approximately 2.0 Å.

The top five disulfide-linked secretin peptides ranked according to these criteria were chosen for experimental testing. A secretin analogue incorporating cysteines and a disulfide bond between residues 4 and 10 was also prepared and tested, based on the position of the disulfide bond naturally occurring in calcitonin in the sequence alignment of Neumann et al.,¹⁵ in which a conserved helix-capping structural motif was proposed as playing an important role in natural ligands for family B G protein-coupled receptors. Each of the peptides in the series, except for Cys^{4,10}-sec also incorporated a tyrosine in position 10 to replace the natural leucine located in that position for possible radioiodination. This residue replacement has previously been shown to be well tolerated for normal secretin binding and biological activity.^{16,17}

The sequences of these peptides ([Cys^{2,7},Tyr¹⁰]rat secretin-27 (Cys^{2,7}-sec), [Cys^{3,5},Tyr¹⁰]rat secretin-27 (Cys^{3,5}-sec), [Cys^{3,6},Tyr¹⁰]rat secretin-27 (Cys^{3,6}-sec), [Cys^{4,7},Tyr¹⁰]rat secretin-27 (Cys^{4,7}-sec), [Cys^{7,9},Tyr¹⁰]rat secretin-27 (Cys^{7,9}-sec), and [Cys^{4,10}]rat secretin-27 (Cys^{4,10}-sec)) are shown in Figure 1,¹⁸ with their structural characteristics as predicted by molecular modeling shown in Table 1. Each of these peptides was able to achieve a conformation with backbone root mean square deviation (RMSD) relative to the reference conformation of receptor-bound PACAP¹³ that was very small. Each peptide had acceptable length disulfide bonds with acceptable dihedral angles, although the energies varied considerably.

Figure 2 illustrates the receptor binding¹⁹ and biological activity²⁰ characteristics of each of the peptides. It shows that neither the linear nor disulfide-bonded forms of Cys^{3,6}-sec and Cys^{4,10}-sec was able to bind to the secretin receptor or to stimulate demonstrable cAMP responses in secretin receptor-expressing CHO-SecR cells. This is also the case for the disulfide-bonded Cys^{2,7}-sec analogue, while its linear form was able to compete for binding of the secretin radioligand and was able to stimulate an almost full cAMP response at its highest concentration of 1 μM. Both linear and disulfide-bonded forms of Cys^{3,5}-sec and Cys^{4,7}-sec were only able to compete for secretin radioligand binding and to stimulate cAMP responses in CHO-SecR cells at their highest concentrations utilized.

Of all the dual cysteine-containing secretin analogues, only disulfide-bonded Cys^{7,9}-sec was able to fully compete for binding of the secretin radioligand to CHO-SecR membranes with an affinity close to that of natural secretin. It had a K_i of 11 ± 3.3 nM, while natural secretin bound with a K_i of 3.5 ± 1.1 nM (Fig. 2). The disulfide-bonded Cys^{7,9}-sec peptide was the only full agonist in this series, stimulating an intracellular cAMP response in CHO-SecR cells not different from that in response to natural secretin. This constrained peptide stimulated cAMP responses in a concentration-dependent manner, with potency similar to secretin (EC₅₀ values: secretin, 80 ± 19 pM; Cys^{7,9}-sec, 100 ± 23 pM). The linear form of this peptide bound to the secretin receptor and stimulated a partial cAMP response in CHO-SecR cells only at its highest concentration tested (1 μM). Figure 3 illustrates the predicted receptor-bound conformation of the disulfide-bonded active peptide, showing that this constrained structure is able to achieve a backbone conformation close to that observed for receptor-bound PACAP.

In this work, we have attempted to gain insights into the conformation of the functionally-important amino-terminal region of secretin, as bound to its receptor. The secretin receptor was the first member of the B family of G protein-coupled receptors to be discovered,²¹ and has become a prototype for this family in every way. This receptor shares all the key signature structural features of members of this family,^{11,12} and the secretin peptide ligand

shares the structural features and structure-activity relationships characteristic of this family.^{11,12} Like other members of this family,²² the amino-terminal region of secretin is critical for its biological activity^{11,12} and elimination of this region by truncation results in antagonist activity.^{23,24}

Despite the functional importance of the amino-terminal region of secretin and other natural agonist ligands of family B G protein-coupled receptors, little is known about the molecular basis of its interaction with these receptors. There are some general spatial approximation data gleaned from photoaffinity labeling studies.^{7–10} However, of necessity, such studies need to utilize bulky, non-natural photolabile moieties for activation and induction of covalent bonds. Because of the critical functional importance of this region of these peptides, incorporation of this type of photolabile group often reduces binding affinity and biological activity of these probes. An interesting approach has recently been applied to the parathyroid hormone receptor in which cysteine scanning with formation of disulfide bonds was utilized to gain less disruptive insights into spatial approximations.²⁵ Even in that setting, spatial approximation of flexible peptides to flexible, non-constrained extracellular loop domains of these receptors can provide only limited value in understanding bound conformations.

It would be ideal to have high resolution NMR or crystal structures of a peptide bound to an intact family B G protein-coupled receptor that would include its helical bundle and loop domains. However, no such structure is yet available. Only recently have structures of intact G protein-coupled receptors been available,^{26–29} and all of these represent family A receptors, a group that is predicted to have clear structural differences from members of family B.^{30,31}

In the absence of such direct insights, we have attempted to constrain the conformation of the amino terminus of secretin analogues using intramolecular disulfide bonds. The linear peptides incorporating the two cysteine residues that we prepared provide insights into whether the specific residues replaced by the cysteines are critical for receptor binding and biological activity. In the absence of activity at the receptor of these linear peptides, constraining their conformation with disulfide bonds may not be additionally informative, since absence of activity of those structures may simply reflect absence of functional groups critical to the pharmacophore. The 3–6 and 4–10 di-cysteine secretin peptides may fall into this category.

When the linear peptides including the cysteine replacements have some activity, representing either receptor binding or biological activity, such as all the other peptides in this series, we can be more confident that the conformational constraints provide useful information. If the disulfide-bonded constrained peptides have less activity than its linear analogue, the constraint is likely not applicable to the receptor-bound peptide. This is the situation for the 2–7, 3–5, and 4–7 di-cysteine secretin peptides. If the constrained peptide has greater activity than its linear analogue, particularly if this activity is similar to that of natural secretin, this supports a highly meaningful constraint, matching the natural binding conformation of the hormone. This is the situation for the 7–9 di-cysteine secretin peptide.

It is noteworthy that molecular modeling was not able to predict which of the first five disulfide-bonded secretin analogues would have optimal activity. It is interesting that the most active peptide of this group had the shortest disulfide bond that would ordinarily be considered less-than-optimal, however, the dihedral angle of this bond was closest to ideal and the energy was reasonably low, indicating that the bond was not unduly strained. Because there is a tradeoff between maintaining the bound PACAP backbone conformation and optimizing the disulfide bond geometry, the disulfide bond strain could be reduced at

the expense of slightly increasing the backbone deviation. The molecular modeling did demonstrate that the peptide with disulfide bond between residues 4 and 10 would not have a conformation similar to receptor-bound PACAP,¹³ but given the disulfide-bonded structure of natural calcitonin, this was still worthwhile to prepare and study.

It is likely that the conformations of receptor-bound secretin and receptor-bound PACAP are similar in the 7–9 region. This site of a tight turn could functionally mimic the helix N-capping motif postulated to be common to natural peptide ligands for family B G protein-coupled receptors.¹⁵ Presumably, both this disulfide bond and the classical N-capping motifs are able to direct the critical amino-terminal functional groups to their ideal docking partners. The absolute level of similarity and differences between the amino-terminal conformations of the various members of this family is still unclear. Only after gaining additional experimental constraints will there be adequate information to compare these important structures.

Acknowledgments

This work was supported by grants from the National Institutes of Health (DK46577) and from the Fiterman Foundation. The authors thank Ms. Mary Lou Augustine for her technical assistance.

References and notes

1. Parthier C, Kleinschmidt M, Neumann P, Rudolph R, Manhart S, Schlenzig D, Fanghanel J, Rahfeld JU, Demuth HU, Stubbs MT. *Proc Natl Acad Sci U S A*. 2007; 104:13942.
2. Pioszak AA, Xu HE. *Proc Natl Acad Sci U S A*. 2008; 105:5034. [PubMed: 18375760]
3. Sun C, Song D, Davis-Taber RA, Barrett LW, Scott VE, Richardson PL, Pereda-Lopez A, Uchic ME, Solomon LR, Lake MR, Walter KA, Hajduk PJ, Olejniczak ET. *Proc Natl Acad Sci U S A*. 2007; 104:7875. [PubMed: 17470806]
4. Grace CR, Perrin MH, Gulyas J, Digruccio MR, Cattle JP, Rivier JE, Vale WW, Riek R. *Proc Natl Acad Sci U S A*. 2007; 104:4858. [PubMed: 17360332]
5. Runge S, Thogersen H, Madsen K, Lau J, Rudolph R. *J Biol Chem*. 2008; 283:11340. [PubMed: 18287102]
6. Pioszak AA, Parker NR, Suino-Powell K, Xu HE. *J Biol Chem*. 2008; 283:32900. [PubMed: 18801728]
7. Bisello A, Adams AE, Mierke DF, Pellegrini M, Rosenblatt M, Suva LJ, Chorev M. *J. Biol. Chem*. 1998; 273:22498. [PubMed: 9712875]
8. Dong M, Lam PC, Pinon DI, Sexton PM, Abagyan R, Miller LJ. *Mol. Pharmacol*. 2008; 74:413. [PubMed: 18467541]
9. Dong M, Li Z, Pinon DI, Lybrand TP, Miller LJ. *J. Biol. Chem*. 2004; 279:2894. [PubMed: 14593094]
10. Dong M, Pinon DI, Cox RF, Miller LJ. *J. Biol. Chem*. 2004; 279:31177. [PubMed: 15155765]
11. Dong M, Miller LJ. *Receptors Channels*. 2002; 8:189. [PubMed: 12529936]
12. Ulrich CD 2nd, Holtmann M, Miller LJ. *Gastroenterology*. 1998; 114:382. [PubMed: 9453500]
13. Inooka H, Ohtaki T, Kitahara O, Ikegami T, Endo S, Kitada C, Ogi K, Onda H, Fujino M, Shirakawa M. *Nat Struct Biol*. 2001; 8:161. [PubMed: 11175907]
14. Abagyan RA, Totrov M. *J Comput Phys*. 1999; 151:402.
15. Neumann JM, Couvineau A, Murail S, Lacapere JJ, Jamin N, Laburthe M. *Trends Biochem Sci*. 2008; 33:314. [PubMed: 18555686]
16. Gardner JD, Conlon TP, Beyerman HC, Van Zon A. *Gastroenterology*. 1977; 73:52. [PubMed: 193759]
17. Kofod H. *Int. J. Pept. Protein Res*. 1991; 37:185. [PubMed: 1651289]
18. All of the peptides were synthesized using standard solid-phase synthesis techniques with Pal resin (Advanced ChemTech, Louisville, KY) and Fmoc-protected amino acids, as described previously

(Powers SP, Pinon DI, Miller LJ. *Int. J. Pept. Protein Res.* 1988; 31:429. [PubMed: 3410633]). After completion of the synthesis of each linear peptide, it was cleaved from the resin using a solution of 6.25% (wt/vol) phenol, 2% (vol/vol) triisopropylsilane, 4% (vol/vol) thioanisole, 4% (vol/vol) distilled water, and 83% (vol/vol) trifluoroacetic acid. This also removed all side chain-protecting groups, including that of *N*- α -Fmoc-*N*- β -S-trityl-L-cysteine. An aliquot of each linear peptide was utilized to prepare its disulfide-bonded form. The bond linking the sulfhydryl groups of the two cysteine residues within each cyclic peptide was formed by air oxidation in the presence of dimethyl sulfoxide. For this, an aliquot of the linear peptide was dissolved in 5% (v/v) acetic acid, had its pH adjusted to approximately 6 with 1 M (NH₄)₂CO₃, and was diluted with water to yield a peptide concentration of 0.5 to 1 mg/ml. While stirring and exposed to the air, dimethyl sulfoxide was added to a final concentration of 10% (v/v). The oxidation process was monitored for completion using reversed-phase HPLC, typically taking between two and five hours. All linear and disulfide-bonded cyclic peptides were purified to homogeneity by reversed-phase HPLC on an octadecylsilane column running a 10 to 60% acetonitrile gradient with a background of 0.1% trifluoroacetic acid. Expected molecular masses of the synthetic products were verified by matrix-assisted laser desorption/ionization-time of flight mass spectrometry.

19. The receptor-binding characteristics of each of the linear and disulfide-bonded secretin analogues were determined using membranes from receptor-bearing CHO-SecR cells (Ulrich CD 2nd, Pinon DI, Hadac EM, Holicky EL, Chang-Miller A, Gates LK, Miller LJ. *Gastroenterology.* 1993; 105:1534. [PubMed: 8224659]) and a competitive radioligand binding assay. Enriched plasma membranes from CHO-SecR cells were prepared using discontinuous sucrose gradient centrifugation (Hadac EM, Ghanekar DV, Holicky EL, Pinon DI, Dougherty RW, Miller LJ. *Pancreas.* 1996; 13:130. [PubMed: 8829180]) and were stored in aliquots in Krebs-Ringers/HEPES (KRH) medium (25 mM HEPES, pH 7.4, 104 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM KH₂PO₄, 1.2 mM MgSO₄) containing 0.01% soybean trypsin inhibitor and 1 mM phenylmethylsulfonyl fluoride at -80 °C until use. Secretin radioligand was prepared by the oxidative radioiodination of [Tyr¹⁰]rat secretin-27 using 1 mCi Na¹²⁵I and exposure to the IODO-BEAD solid-phase oxidant (Pierce Chemical Co, Rockford, IL) for 15 s in 0.1 M borate buffer (pH 9). This was purified using reversed-phase HPLC to yield a specific radioactivity of approximately 2,000 Ci/mmol (Powers SP, Pinon DI, Miller LJ. *Int. J. Pept. Protein Res.* 1988; 31:429. [PubMed: 3410633]). In brief, approximately 5–10 μ g of CHO-SecR membranes were incubated with a constant amount of secretin radioligand (approximately 20,000 rpm) and increasing concentrations of secretin or one of its analogues (from 0 to 1 μ M) in KRH medium containing 0.01% soybean trypsin inhibitor, 1 mM phenylmethylsulfonyl fluoride and 0.2% bovine serum albumin for 1 h at room temperature (reaction volume, 500 μ l). Separation of bound from free radioligand was performed by centrifugation at 14,000 rpm at 4 °C, and washing twice with ice-cold KRH medium. Bound radioactivity was quantified with a γ -spectrometer. Non-saturable binding was determined in the presence of 1 μ M secretin and represented less than 15% of total radioligand bound. Binding kinetics were determined by analysis with the LIGAND program of Munson and Rodbard (Munson PJ, Rodbard D. *Anal. Biochem.* 1980; 107:220. [PubMed: 6254391]).
20. The biological activity of each of the secretin analogues was determined by examining its ability to stimulate cAMP responses in secretin receptor-expressing CHO-SecR cells grown in 96-well plates. After cells were grown (~8,000 cells per well) for two days, they were washed with PBS and stimulated with increasing concentrations of secretin or its analogues (0 to 1 μ M) in KRH medium containing 0.01% soybean trypsin inhibitor, 0.2% bovine serum albumin, 0.1% bacitracin, and 1 mM 3-isobutyl-1-methylxanthine for 30 min at 37 °C. Cells were then lysed with 6% ice-cold perchloric acid for 15 min with vigorous shaking and the lysates were adjusted to pH 6 with 30% NaHCO₃. The cAMP levels in the lysates were assayed in a 384-well white Optiplate using a LANCE kit from PerkinElmer (Boston, MA).
21. Ishihara T, Nakamura S, Kaziro Y, Takahashi T, Takahashi K, Nagata S. *EMBO J.* 1991; 10:1635. [PubMed: 1646711]
22. Mayo KE, Miller LJ, Bataille D, Dalle S, Goke B, Thorens B, Drucker DJ. *Pharmacol. Rev.* 2003; 55:167. [PubMed: 12615957]
23. Gardner JD, Conlon TP, Fink ML, Bodanszky M. *Gastroenterology.* 1976; 71:965. [PubMed: 186350]

24. Robberecht P, Conlon TP, Gardner JD. *J. Biol. Chem.* 1976; 251:4635. [PubMed: 181379]
25. Monaghan P, Thomas BE, Woznica I, Wittelsberger A, Mierke DF, Rosenblatt M. *Biochemistry.* 2008; 47:5889. [PubMed: 18459800]
26. Cherezov V, Rosenbaum DM, Hanson MA, Rasmussen SG, Thian FS, Kobilka TS, Choi HJ, Kuhn P, Weis WI, Kobilka BK, Stevens RC. *Science.* 2007; 318:1258. [PubMed: 17962520]
27. Jaakola VP, Griffith MT, Hanson MA, Cherezov V, Chien EY, Lane JR, Ijzerman AP, Stevens RC. *Science.* 2008; 322:1211. [PubMed: 18832607]
28. Scheerer P, Park JH, Hildebrand PW, Kim YJ, Krauss N, Choe HW, Hofmann KP, Ernst OP. *Nature.* 2008; 455:497. [PubMed: 18818650]
29. Warne T, Serrano-Vega MJ, Baker JG, Moukhametzianov R, Edwards PC, Henderson R, Leslie AG, Tate CG, Schertler GF. *Nature.* 2008; 454:486. [PubMed: 18594507]
30. Donnelly D. *FEBS Lett.* 1997; 409:431. [PubMed: 9224704]
31. Tams JW, Knudsen SM, Fahrenkrug J. *Receptors & channels.* 1998; 5:79. [PubMed: 9606712]

Figure 1.

Primary structures of secretin analogues used in this study. Shown are the amino acid sequences of natural rat secretin and its dual cysteine-containing analogues. Natural residues are illustrated in grey, while modified residues are illustrated in black. Disulfide bonds linking the side chains of the cysteine residues are illustrated with dotted lines.

Figure 2.

Binding and biological activities of the dual cysteine-containing secretin analogues. *Left column*, curves reflecting the ability of increasing concentrations of secretin and secretin analogues (linear and disulfide-bonded forms) to compete for binding of the secretin radioligand to CHO-SecR membranes. Values illustrated represent percentages of saturable binding, expressed as the means $\pm$ S.E.M. of duplicate values from a minimum of three independent experiments. *Right column*, intracellular cAMP responses to increasing concentrations of these peptides in CHO-SecR cells. Data points represent the means $\pm$ S.E.M. of three independent experiments performed in duplicate, normalized relative to the maximal responses of these cells to secretin. Basal and maximal cAMP levels stimulated by secretin were 3.7 ± 0.7 and 209 ± 48 pmol/million cells, respectively.

Figure 3.

Molecular modeling of the amino-terminal region of disulfide-bonded Cys^{7,9}-sec. Shown are the molecular modeling results for the amino-terminal portion of the secretin peptide with a disulfide bond between cysteine residues in positions 7 and 9, which had the highest observed biological activity and binding affinity in the experimental assays. The backbone of the PACAP peptide structure (PDB entry 1GEA), used to constrain the disulfide-bonded secretin peptide, is shown in green. This structure shows that the disulfide-bonded secretin peptide is able to maintain reasonable disulfide bond geometry with a backbone conformation close to that observed for receptor-bound PACAP.

Table 1

Molecular modeling simulation results for the disulfide-bonded secretin analogues studied.

Positions of cysteine residues within secretin analogues	Disulfide bond length (Å)	Disulfide bond dihedral angle (degrees)	Energy (kcal/mol)	Backbone RMSD from receptor-bound PACAP structure (Å)
2–7	2.02	102	–31.6	0.20
3–5	1.90	96	–23.7	0.24
3–6	1.92	102	–3.63	0.20
4–7	2.06	98	–15.3	0.32
7–9	1.66	85	–6.97	0.28
4–10	1.91	55	–38.8	0.20

Shown are disulfide bond characteristics of disulfide-bonded secretin analogues, along with their backbone deviations from the structure of receptor-bound PACAP.¹³