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Insights into the impact of phenolic residue incorporation at each position along secretin for receptor binding and biological activity

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ABSTRACT

Understanding of the structural importance of each position along a peptide ligand can provide important insights into the molecular basis for its receptor binding and biological activity. This has typically been evaluated using serial replacement of each natural residue with an alanine. In the current report, we have further complemented alanine scanning data with the serial replacement of each residue within secretin-27, the natural ligand for the prototypic class B G protein-coupled secretin receptor, using a photolabile phenolic residue. This not only provided the opportunity to probe spatial approximations between positions along a docked ligand with its receptor, but also provided structure–activity insights when compared with tolerance for alanine replacement of the same residues. The pattern of sensitivity to phenolic residue replacement was periodic within the carboxyl-terminal region of this peptide ligand, corresponding with alanine replacements in that region. This was supportive of the alpha-helical conformation of the peptide in that region and its docking within a groove in the receptor amino-terminal domain. In contrast, the pattern of sensitivity to phenolic residue replacement was almost continuous in the amino-terminal region of this peptide ligand, again similar to alanine replacements, however, there were key positions in which either the phenolic residue or alanine was differentially preferred. This provided insights into the receptor environment of the portion of this ligand most critical for its biological activity. As the structure of the intact receptor is elucidated, these data will provide a guide for ligand docking to the core domain and to help clarify the molecular basis of receptor activation.

1. Introduction

Replacement of each residue along a peptide ligand with an alanine residue represents a classical approach to determine the importance of each residue in hormone function, in making contributions to both receptor binding and biological activity [1–5]. Alanine has been chosen for this, based on it being the smallest of the uncharged non-polar amino acid residues that maintains the integrity of the peptide backbone, but that does not contribute to higher energy charge–charge interactions or strong hydrophobic interactions of more specialized residues. However, the usefulness of an alanine-scanning data set may be limited. Loss-of-function of a particular alanine-replacement mutant has been interpreted to suggest that the replaced residue is critical to function and might be difficult to replace in a peptide analogue. However, in preparing intrinsic photolabile probes for photoaffinity labeling of a hormone receptor, we have observed examples of successful incorporation of a bulky, hydrophobic photolabile moiety into a peptide position that poorly tolerates alanine replacement [6–8].

In the current work, we have focused on the natural peptide ligand for the secretin receptor, a prototypic class B peptide hormone-binding G protein-coupled receptor [9]. This receptor family typically binds and is activated by moderate length peptides having an extended pharmacophoric region, with the carboxyl-terminal region of the hormone contributing binding affinity to the structurally distinct, disulfide-bonded receptor amino terminus and the amino-terminal region of the hormone contributing biological activity and additional binding affinity, likely directed toward the core regions of the receptor [10]. Indeed, these features have been shown to be shared by secretin, a 27-residue peptide [11]. We previously reported the replacement of each secretin residue with alanines and demonstrated that the pattern of functional importance closely followed that of related peptide hormone receptors in this family [2]. We have also replaced sixteen natural residues along secretin with photolabile benzoyl-phenylalanine (Bpa) or benzoyl-benzoyl-lysine ((BzBz)Lys) for site-specific photoaffinity labeling studies of this receptor [6–8,11–18]. In such studies, it is important for the photolabile hormone analogues to maintain adequate binding affinity and full biological activity to be certain that the probe occupy a site similar to that occupied by the natural hormone. In this experience, we have described three secretin analogues in which the photolabile moiety was successfully incorporated into a position in which alanine replacement has been poorly tolerated (positions 6, 22, and 26) [6–8].

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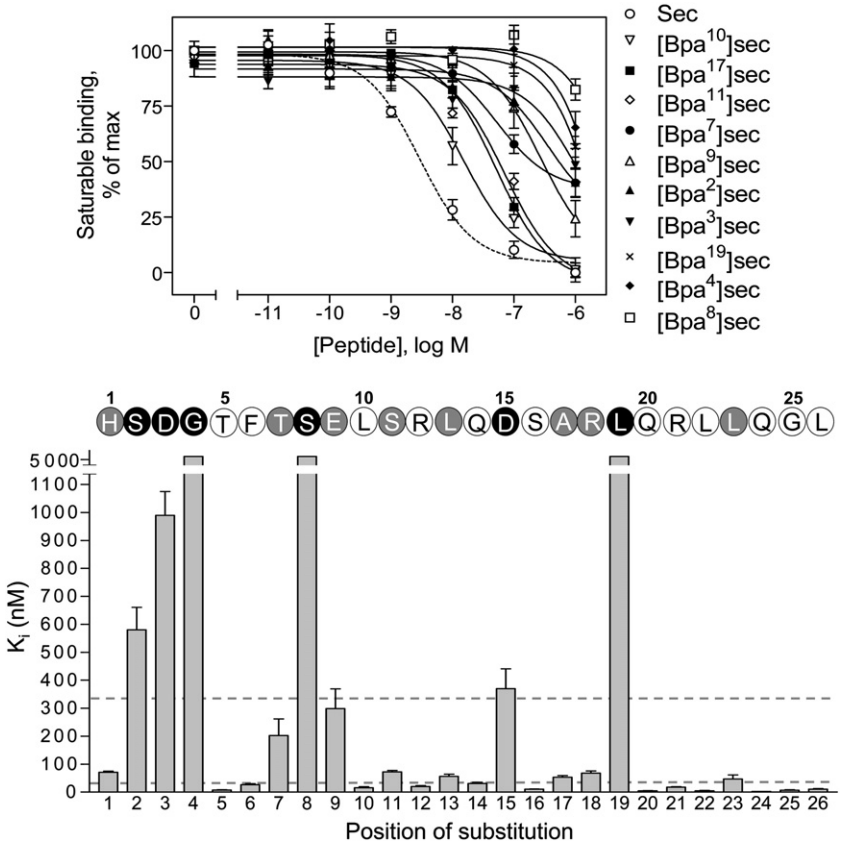

Fig. 1. Binding activities of phenolic-replacement analogues of secretin. Top, binding curves of increasing concentrations of secretin and phenolic-replacement analogues to compete for binding of secretin radioligand to CHO-SecR cells. Values illustrated represent saturable binding as percentages of maximal binding observed in the absence of the competing peptide and are expressed as the means ± S.E.M. of duplicate values from a minimum of three independent experiments. The rank order of affinities from highest to lowest is shown on the right of the graph (top to bottom). Bottom, role of each residue in secretin on binding to its receptor. Shown are the K_i values of each of the secretin analogues incorporating a phenolic residue and the secretin sequence illustrating the role of each residue in binding to the secretin receptor. Open circles represent residues whose replacement by phenolic residues resulted in less than 10-fold in binding affinity comparing to natural secretin. Gray and black circles represent residues whose replacement with phenolic residues resulted in more than 10-fold but less than 100-fold, and more than 100-fold increase in binding affinity (dashed lines), respectively, comparing to natural secretin.

We have, therefore, now prepared twelve additional analogues of secretin to complete the series of analogues with phenolic residue incorporations throughout the pharmacophore (every position from 1 through 26) and to compare this series with the alanine-replacement series, exploring how such data might provide insights into the molecular mechanism of peptide docking and receptor activation.

2. Methods

2.1. Reagents

Fmoc-protected amino acids for peptide synthesis were purchased from Advanced ChemTech (Louisville, KY) and Pal resin was from Sigma-Aldrich (St. Louis, MO). ^{125}Iodine was from PerkinElmer Life Sciences (Boston, MA). Dulbecco's modified Eagle's medium (DMEM) and soybean trypsin inhibitor were from Invitrogen (Carlsbad, CA). Fetal Clone II culture medium supplement was from Hyclone laboratories (Logan, UT). Bovine serum albumin was from Serologicals Corp. (Norcross, GA). All other reagents were analytical grade.

2.2. Peptide synthesis

Eleven rat secretin peptide analogues were prepared for the current study, ten of which incorporated a Bpa into positions 2–4, 7–11, 17, and 19 and one of which incorporated a *p*-nitro-phenylalanine (NO_2-Phe) in position 15. Each probe (except for position 10 Bpa probe) incorporated a tyrosine residue in position 10 to replace the natural leucine residue in that position as a site for radioiodination that has previously been well tolerated [19,20]. For the probe incorporating a Bpa in position 10, a tyrosine for radioiodination was incorporated in position 26. All these peptides were synthesized using manual solid-phase synthesis techniques with Pal resin and Fmoc-protected amino acids and purified by reversed-phase HPLC using procedures described previously [21]. Expected molecular masses were verified by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry.

2.3. Radioiodination

The radioligand was prepared as described previously by oxidative radioiodination of [Tyr^{10}]rat secretin-27 using 1 mCi Na^{125}I and exposure for 15 s to the solid phase oxidant, Iodo-beads [21]. The radioiodinated peptide was purified by reversed-phase HPLC to yield specific radioactivity of ~2000 Ci/mmol [21].

2.4. Receptor source

A CHO cell line stably expressing the wild type rat secretin receptor (CHO-SecR) that had been previously established was used as source of receptor for the current study [22]. These cells were cultured in Ham's F-12 medium supplemented with 5% Fetal Clone II in a humidified atmosphere containing 5% CO_2 at 37 °C, and they were passaged approximately twice per week.