Communications to the Editor

Design of Potent, Cyclic Peptide Bradykinin Receptor Antagonists from Conformationally Constrained Linear Peptides

Sarvajit Chakravarty, Deidre Wilkins, and Donald J. Kyle*

Scios Nova Inc., 6200 Freeport Centre, Baltimore, Maryland 21224

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I. Introduction. Over the past several years, at least two generations of peptide bradykinin receptor antagonists have been reported. These include the prototypical first generation antagonist, NPC 567 (D-Arg[0]-Arg[1]-Pro[2]-Hyp[3]-Gly[4]-Phe[5]-Ser[6]-D-Phe[7]-Phe[8]-Arg[9]),[1] and the more potent second generation antagonists HOE 140 (D-Arg[0]-Arg[1]-Pro[2]-Hyp[3]-Gly[4]-Thi[5]-Ser[6]-D-Tic[7]-Oic[8]-Arg[9]) and NPC 17731 (D-Arg[0]-Arg[1]-Pro[2]-Hyp[3]-Gly[4]-Phe[5]-Ser[6]-[D-Hype(trans propyl)][7]-Oic[8]-Arg[9]).[2-4] Although the latter are potentially exciting therapeutic agents,[5-9] their widespread use could ultimately be limited by poor oral activity, short duration of action, and high cost of manufacturing. Therapeutically, one would be most interested in a non-peptide antagonist which might overcome many, if not all, of these shortcomings. Despite massive efforts in many synthetic laboratories as well as exhaustive random screening, no potent, selective, and competitive non-peptide bradykinin receptor antagonists have yet been reported. In the absence of such a series of small, structurally dissimilar, molecules upon which to formulate a structure-activity relationship, one alternative strategy is the direct conversion of a second generation peptide antagonist into a non-peptide antagonist in a systematic fashion. Although this has been accomplished recently in situations where a non-peptide "lead" molecule with modest biological activity was optimized via a parallel peptide series,[10-12] there are no examples describing the *ad hoc* conversion of a peptide antagonist into a nonpeptide antagonist.

As a prelude to establishing the synthetic framework needed for this approach, knowledge of the relative importances of individual amide bonds and side chain groups as well as three-dimensional backbone conformation is required. We are currently investigating each of these three aspects but report here early results which provide insight into the backbone conformation of a prototypical second generation bradykinin receptor antagonist in the biologically active state. This structural insight led to the preparation of two cyclic peptide bradykinin antagonists, of which there are no prior examples.

This study is comprised of a series of second generation peptide antagonists containing the well known backbone constraint(s) *N*- and/or C^{α}-methyl, incorporated at position(s) Gly[4], Phe[5], or both, in the peptide D-Arg[0]-Arg[1]-Pro[2]-Hyp[3]-Gly[4]-Phe[5]-Ser[6]-D-Tic[7]-Oic[8]-Arg[9]. *N*-methyl substitution in the backbone of an L-amino acid is known to disfavor helical, or twisted, backbone conformations while favoring an extended backbone.[13,14] The contrasting C^{α}-methyl modification tends to favor a helical (twisted), rather than extended, conformation.[13,14] These conformational preferences apply only to the backbone ϕ,ψ angles (where ϕ_i and ψ_i correspond to backbone dihedral angles for residue i defined by the four adjacent amino acid backbone atoms C_{i-1}-N_i-C^{α}_i-C_i and N_i-C^{α}_i-C_i-N_{i+1}, respectively) of the amino acid residues bearing the modification. With the exception of the C-terminus,[15,19,20] there have been no conformational investigations reported for any other sections of the new, second generation peptides.

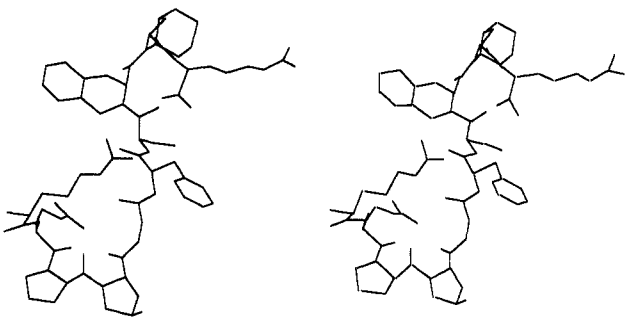

Figure 1. Stereoview of the backbone and side chain atoms in the peptide D-Arg[0]-Arg[1]-Pro[2]-Hyp[3]-Gly[4]-Phe[5]-Ser[6]-D-Tic[7]-Oic[8]-Arg[9]. The backbone dihedral angles of the C-terminal residues Ser[6] through Arg[9] are set to values corresponding to those determined by previous computational and NMR studies.[19,20] The ϕ, ψ dihedral angles for Phe[5] are set at −60°, −60° as they are proposed to be on the basis of the binding afinity of the C^{α}-methyl Phe[5]-containing decapeptide, VI.

II. Methods. A. Peptide Synthesis. All peptides were synthesized manually using *tert*-butyloxycarbonyl amino acids.[21] Boc-Oic-OH was prepared according to previously reported procedures.[22] Boc-L-(α-methyl)phenylalanine was prepared by treating the corresponding amino acid with di-*tert*-butyl dicarbonate, according to standard protocol.[23] Boc-Arg(Tos) substituted PAM resin (loading 0.6 mmol/g) was purchased from Applied Biosystems Inc. (Foster City, CA) and used for the purpose. Amino acids were introduced into the growing peptide chain, according to the amino acid sequence using single diisopropylcarbodiimide/hydroxybenzotriazole hydrate mediated couplings according to standard procedures. The finished peptidyl resins were treated with anhydrous hydrogen fluoride (10 mL per gram of peptidyl-resin) in the presence of 10% anisole (scavenger), and the crude peptides were purified by reverse phase high performance liquid chromatography, on a C_{18} column, using a gradient of 5–70% water/acetonitrile (containing 0.1% trifluoroacetic acid). The purity of the peptides was determined by analytical reverse phase HPLC (using linear a gradient of 5–80% water/acetonitrile, containing 0.1% trifluoroacetic acid), fast atom bombardment mass spectroscopy, and amino acid analysis.

B. Modeling Procedure. All calculations were performed using the program CHARMm, Version 22 (Molecular Simulations, Inc.) on a Silicon Graphics computer workstation. Results were graphically rendered on the workstation using QUANTA 3.3 (Molecular Simulations, Inc.). The structure shown in Figure 1 was generated by adjusting the ϕ and ψ backbone dihedral angles for the

* Corresponding author.

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Table I. Peptide Series Containing *N*- and/or C^{α}-Methyl Substitutions in Residues Gly[4] and/or Phe[5]

peptide	amino acid sequence										K_i[a] (nM)	pA_2[b]
	Parent Peptide											
I	D-Arg	Arg	Pro	Hyp	Gly	Phe	Ser	D-Tic	Oic	Arg	0.08	8.82
	N-Methyl Substituted Gly[4] or Phe[5] Peptides											
II	D-Arg	Arg	Pro	Hyp	*N*-methyl Gly	Phe	Ser	D-Tic	Oic	Arg	30.77 ± 15.49	
III	D-Arg	Arg	Pro	Hyp	Gly	*N*-methyl Phe	Ser	D-Tic	Oic	Arg	26.45 ± 10.05	5.60
IV	D-Arg	Arg	Pro	Hyp	*N*-methyl Gly	*N*-methyl Phe	Ser	D-Tic	Oic	Arg	1693.33 ± 26.03	
	C^{α}-Methyl Substituted Gly[4] or Phe[5] Peptides											
V	D-Arg	Arg	Pro	Hyp	C^{α}-methyl Gly	Phe	Ser	D-Tic	Oic	Arg	82.30	
VI	D-Arg	Arg	Pro	Hyp	Gly	C^{α}-methyl Phe	Ser	D-Tic	Oic	Arg	0.54	7.9
VII	D-Arg	Arg	Pro	Hyp	C^{α}-methyl Gly	C^{α}-methyl Phe	Ser	D-Tic	Oic	Arg	3030	

[a] K_i values were determined in guinea pig ileum againt [^{3}H]bradykinin following previously described methods.[16] [b] pA_2 values were determined in guinea pig ileal tissues as reported elsewhere.[24]

Table II. Cyclic Peptide Bradykinin Receptor Antagonists Designed To Enforce a Helical Twist about Phe[5]

peptide	amino acid sequence	K_i[a] (nM)	pA_2[b]
VIII	D-Arg-Arg-Cys-Pro-Gly-Cys-Ser-D-Tic-Oic-Arg	1.5 ± 0.1	6.62
IX	D-Arg-Arg-Cys-Pro-Gly-Phe-Cys-D-Tic-Oic-Arg	14.83 ± 3.42	6.3

[a] K_i values were determined in guinea pig ileum against [^{3}H]bradykinin following previously described methods.[16] [b] pA_2 values were determined in guinea pig ileal tissues as reported elsewhere.[24]

C-terminal residues Ser-D-Tic-Oic-Arg to values previously observed in solution by NMR at 600 MHz.[20] The backbone dihedral angles about Phe[5] were adjusted to −60°, −60° in accordance with the affinity results obtained for the peptide bearing the C^{α}-methyl constraint at Phe[5]. The geometry of the initial structure was optimized via 200 steps of Adopted-Basis-Newton-Raphson energy minimization.

III. Results and Discussion. Initially, six decapeptides were prepared, each related to a common parent structure, I (D-Arg[0]-Arg[1]-Pro[2]-Hyp[3]-Gly[4]-Phe[5]-Ser[6]-D-Tic[7]-Oic[8]-Arg[9]) which is a potent competitive bradykinin receptor antagonist (K_i = 0.08 nM, pA_2 = 8.8; Table I). The six peptides (II-VII) differ from peptide I only in that they have incorporated *N*- and/or C^{α}-methyl substitution into residue(s) Gly[4], Phe[5], or both. Table I lists this series of peptides, together with B2 receptor affinities as measured by K_i and pA_2 values. The former were determined against [^{3}H]bradykinin in guinea pig ileal membrane preparations as described previously,[16] and the latter were measured in guinea pig ileal tissue following conventional methods.[17] This tissue is known to express only B2 receptors.[18]

With the exception of peptide VI in the series of linear peptides, all linear peptides caused a significant, at least 1000-fold, loss in binding affinity with respect to the unconstrained parent peptide (Table I). Aside from the conformational impact of the *N*-methyl substitution, another possibility is that the removal of a key hydrogen bond donor in this way may have an adverse effect on receptor affinity. The C^{α}-methyl Phe[5] substitution of peptide VI is well tolerated by the receptor as evidenced by only a 7-fold loss in receptor affinity (K_i = 0.54 nM) with respect to peptide I. This implies that the ϕ, ψ backbone dihedral angles about Phe[5] are in the vicinity of −60°,−60° in the biologically active conformation. This represents a helical twist or "kink" in the midsection of the peptide. Adjusting the two backbone dihedral angles about Phe[5] accordingly while imposing the β-turn recently described for the remainder of the C-terminal residues (Ser[6]-D-Tic[7]-Oic[8]-Arg[9]) in aqueous solution,[19,20] results in the proposed bio-active conformation corresponding to peptide I shown in Figure 1.

From these results, two cyclic peptides (VIII and IX) were designed. Via covalent cyclization through the disulfide bond, these two peptides dictate a tight kink about the midportion of the peptide, thereby testing the hypothesis posed by the results obtained for peptides II-VII. Shown in Table II are the K_i's and pA_2's corresponding to peptides VIII and IX. These results indicate that the conformation of each of the two highly constrained cyclic peptide antagonists is compatible with the receptor geometry given the respective K_i's of 1.5 nM and 14.8 nM against [^{3}H]bradykinin binding. The loss in receptor affinity for each cyclic peptide (18-fold for VIII and 180-fold for IX) with respect to the linear parent peptide (I) might be partly attributed to the added steric bulk of the disulfide bond, which may not be completely tolerated by the receptor. Regardless, prior to this report, there have been no disclosures of potent, cyclic peptide bradykinin receptor antagonists. Because of the small size of these cyclized peptides, they are well suited for structure-based two-dimensional NMR studies. This work is currently being pursued in our laboratories and is expected to provide new insight into the overall bioactive conformation of related peptide antagonists.

In conclusion, we are reporting eight new peptides, two of which represent the first examples of potent, cyclic peptide bradykinin receptor antagonists. These cyclized peptides were designed on the basis of the *in vitro* affinity profiles of a small series of linear peptides, each of which contained backbone constraints capable of introducing a predictable geometry about Gly[4] or Phe[5] in the peptide D-Arg[0]-Arg[1]-Pro[2]-Hyp[3]-Gly[4]-Phe[5]-Ser[6]-D-Tic[7]-Oic[8]-Arg[9]. Overall, the results favor the hypothesis that the middle portion of this prototypical, second generation peptide antagonist adopts a helical twist or kink upon complexation with the receptor. This is supported further by the high affinity of the two cyclic peptides, VIII and IX. Admittedly, final verification of this proposal must be derived from X-ray diffraction studies on the ligand–receptor complex. However, in the absence of such information, the peptides presented here represent a valuable surrogate.

Ultimately, one might be able to derive biologically relevant, 3D structural information about these cyclic peptides via NMR experiments. Such information, when

considered together with knowledge about the relative importances of amide bonds and amino acid side chains during receptor binding, could form the basis for the *ad hoc* design of non- or pseudo-peptide antagonists of the bradykinin receptor. This strategy is currently being pursued in our laboratories.

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