Template-Constrained Somatostatin Analogues: A Biphenyl Linker Induces a Type-V′ Turn

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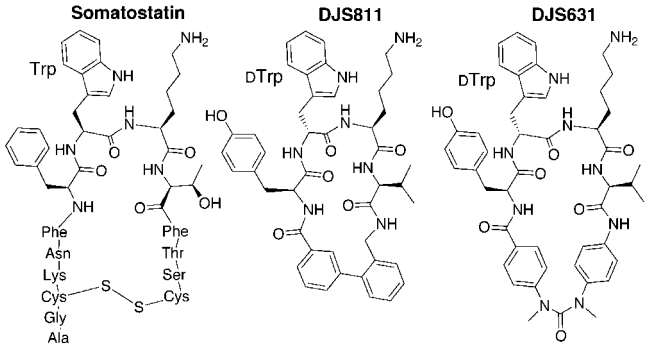
Reverse turns are important for the folding and molecular recognition of proteins and peptides.[1] Thus, there has been interest in stabilizing various turns to provide conformational rigidity and improved bioavailability, while retaining or even enhancing the potency and selectivity of the parent peptide. Macrocyclization has been an effective approach for restricting the conformation of a turn sequence.[1−5] One of the simplest strategies involves backbone cyclization[1,3,5c,f,l] with the incorporation of heterochiral sequences[1,3a,b] such as D-Pro-Pro[3a,b] and peptoids.[5f] This concept has been expanded further by incorporating nonpeptidic linkers[4,5b,d,g,h,k,n] such as dipeptide turn mimetics,[6] which provide extra rigidification and reduced peptidic character. Although these cyclic peptides are fairly rigid, receptor-induced conformational change cannot be completely ruled out.[3c,7] Nevertheless, structures of these cyclic peptides have been important for revealing structure−activity relationships.[2−4b,5b,d,g,h,k,n,7] Previously, we incorporated two linkers into somatostatin analogues that elicit high activity and subtype selectivity (DJS811 and DJS631).[5b] Herein, we report the conformational analysis of these two somatostatin analogues.



Somatostatin,[8a,b] a disulfide-linked 14-residue cyclic peptide, is important for regulating hormone release (growth hormone, glucagon, insulin, gastrin), and for neural transmission. Various somatostatin receptor subtypes for mediating the different biological activities have been identified.[8c,d] The four central residues, Phe-Trp-Lys-Thr, are essential for activity[5n] and appear to form a β-turn (probably type-I β-turn[1,9]) in the native peptide. The replacement of Trp with D-Trp results in peptides with enhanced activity and tunable specificity profiles.[5,8] This D-Trp-Lys heterochiral sequence forms a type-II′ β-turn,[5,9] which is more stable than the type-I β-turn conformation.[1] Streamlined cyclic somatostatin analogues with a type-II′ β-turn have been investigated by the groups of Veber and Hirschman,[5n,o] van Binst,[5k] Goodman,[5f] Kessler,[5g] Hruby,[7a] and others,[5] which have been recently reviewed.[5a] The conformational analyses of these somatostatin analogues have provided the structural basis for bioactivity. In the bioactive type-II′ turn conformation, the side chains of Lys and Trp are in close proximity, which causes a characteristic upfield shift of the Lys γ protons due to the ring current effect of the Trp indole ring. More recently, there has also been development of nonpeptidic compounds that exhibit somatostatin activity and subtype selectivity.[10] In this communication, the structural characterization of subtype-selective somatostatin ana-

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(9) The backbone dihedrals (ϕ_{i+1}, ψ_{i+1}, ϕ_{i+2}, ψ_{i+2}) determine the turn types: I (−60, −30, −90, 0), II′ (60, −120, −80, 0), and V′ (80, −80, −80, 80).

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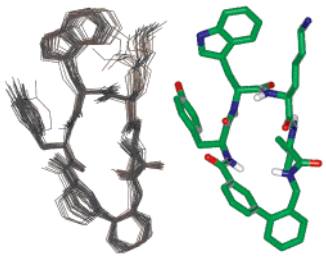

Figure 1. The conformation of DJS811 as determined by ROE-based distance-restrained simulated-annealing. (Left) Superimposition of the 32 converged structures. (Right) Structure closest to the average structure.

Table 1. Dihedral Angles for the Converged Structure of DJS811

residue	ϕ	ψ	χ_1
Tyr	72 ± 3	136 ± 3	-175 ± 5
D-Trp	99 ± 7	-93 ± 5	145 ± 5
Lys	-95 ± 5	93 ± 2	-66 ± 6
Val	-63 ± 6	117 ± 6	179 ± 1

logues DJS811 and DJS631 reveals a novel conformation: the first reported example of a type-V′ β-turn.[1,9]

Sequence specific assignments[11a] were obtained for DJS811 and DJS631 based on TOCSY,[11b] DQF-COSY,[11c] ECOSY,[11d] and ROESY[11e] spectra collected on water/D_2O and D_2O samples. For both compounds, the upfield shift of the Lys γ protons indicative of a bioactive turn conformation was observed (DJS811, 0.305 ppm; DJS631, 0.23 ppm). For DJS811, the Lys β protons and Val γ methyl groups were stereospecifically assigned. The structures were calculated by simulated-annealing protocols[11f] with ROE-derived distance restraints.[12] The more potent compound (DJS811) converged to a single conformation (Figure 1), while the less bioactive compound (DJS631) appeared to adopt at least six conformations. The multiple conformations for DJS631 were evident in the two phenyl rings of the urea template. Although all phenyl ring protons were individually assigned, multiple conformations were necessary to account for all the corresponding ROESY cross-peaks. Since DJS811 exhibits higher bioactivity and a single converged conformation, suggesting the constraints imposed by the linkers promote the bioactive conformation, detailed conformational analysis of DJS811 was pursued.

For DJS811, 42 unique distance restraints were included in the structure calculations (2 medium range, 11 sequential). DQF-COSY spectra revealed average values for $^3J_{HN C\alpha H}$, and ECOSY spectra provided the $^3J_{\alpha\beta}$ values to give two side chain dihedral restraints for two β protons (one each for Val and Lys).[12] Thirty two of the 50 structures converged to a single conformation (Figure 1, Table 1), and the average pairwise heavy atom and backbone[13] rmsd values were 0.82 ± 0.10 and 0.26 ± 0.07 Å, respectively. The rigid backbone conformation may be attributed to the biphenyl turn mimetic. Strikingly, the expected type-II′ β-turn was observed in only six of the 50 structures (for the D-Trp-Lys heterochiral sequence), while the type-V′ β-turn was observed in 32 structures (Table 1). Both conformations are equally

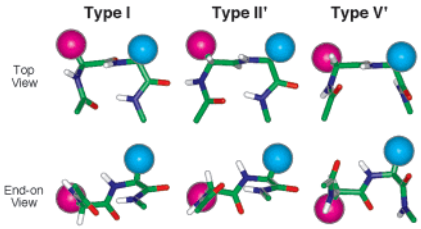

Figure 2. Ideal models of Ac-Ala1/D-Ala1-Ala2-NHMe in various turn conformations. The Cβ atoms are colored in magenta and cyan for residues 1 and 2, respectively. The hydrogens are not shown for clarity except for the CαH of residue 1 and all amide hydrogens.

consistent with the NMR-derived restraints, but the structures with the type-V′ β-turn represent lower-energy conformers than those with the type-II′ β-turn.[12] Additionally, the lack of strong intramolecular hydrogen bonds for the amides in the type-V′ β-turn structures is more consistent with the high variable temperature coefficients for all the amide protons ($-\Delta\delta/\Delta T > 5$ ppb/K).[12] The type-V′ β-turn has been mentioned only as a possibility by Scheraga and co-workers,[1b] but it has not yet been reported in proteins or peptides.[14] Besides exhibiting this unexpected turn type, the Tyr adopts a left-handed conformation ($\phi > 0$), which is generally unfavorable for natural L-amino acids. Attempts to force this residue to a more conventional conformation ($\phi < 0°$) resulted in violations of NMR restraints. Thus, this conformation may serve to stabilize the type-V′ turn or destabilize the type-II′ turn. The unusual backbone conformations may be due to the constraints imposed by the semirigid turn mimetic. Nevertheless, the side chain conformations of D-Trp and Lys are consistent with the established pharmacaphore for somatostatin activity,[5] which explains the bioactivity. The combination of bioactive side chain conformations with subtle changes in the backbone structure may explain the high subtype selectivity for DJS811.

These data illustrate how similar but nonidentical peptides can adopt distinct backbone conformations that present nearly identical constellations of amino acid side chains (Figure 2). The interplay of the plasticity of the amide backbone with the rigidity of an artificial linker allows tuning of the conformation and hence receptor selectivity. In the pioneering development of somatostatin analogues, the selectivity profile was altered by replacing the type-I β-turn with a heterochiral type-II′ β-turn (Figure 2).[5,8] For DJS811, a semirigid biphenyl linker induced a novel structure, the type-V′ β-turn,[1,9] and high receptor selectivity.[5b] Thus, introduction of semirigid linkers into cyclic peptides appears to be a successful strategy for tuning conformational and biological properties.

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Supporting Information Available: Experimental procedures, chemical shift assignments, and coupling constants (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(13) The backbone includes the biphenyl moiety.

(14) The type-V′ β-turn is essentially a compound (classical γ)−(inverse γ) turn (also referred to as $\gamma_c\gamma_i(i, i+1)$ or $\gamma_c(i, i+2) - \gamma_i(i, i+2)$), which was not found in a recent survey by Guruprasad and co-workers: Guruprasad, K.; Prasad, M. S.; Kumar, G. R. *J. Pept. Res.* **2000**, *56*, 250.