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CONFORMATIONAL ENERGY STUDIES OF THE GROWTH HORMONE INHIBITOR, cyclo(Aha-Cys-Phe-D-Trp-Lys-Thr-Cys)

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Summary

The conformation of the molecule cyclo(Aha-Cys-Phe-D-Trp-Lys-Thr-Cys) was studied by empirical conformational energy calculations. The low-energy structure found contains a type II' bend centered at the D-Trp-Lys residues. The lowest energy conformer has the aromatic ring of DTrp positioned such that the γ-protons of the Lys side-chain are in the shielding region (i.e., perpendicular to the center of the aromatic ring). This is in agreement with the NMR results. A mechanism of action for the inhibition of GH release is presented which suggests a conformational change occurs in the D-Trp side-chain ring upon binding to the receptor. The resulting structure has the Phe-D-Trp ring-ring stacking suggested to be responsible for binding and agonist activity of model growth-hormone releasing peptides.

Introduction

In searching for active analogs of the indogenous growth hormone release inhibiting hormone; somatostatin, it was found (1) that a small bicyclic analog cyclo(Aha-Cys-Phe-D-Trp-Lys-Thr-Cys) (I) was more potent in inhibiting the release of GH, Insulin and Glucagon than somatostatin (1) (Aha is ω-aminoheptanoic acid). This analog, which contains only residues 7-10 of somatostatin, is sufficiently small that rigorous empirical conformatioal energy calculations can be carried out to find low-energy structures. Further, the disulfide bridge imparts a conformational constraint which reduces the number of possible conformers considerably. The carba-bridge (6 CH_2's) also restricts the number of allowed conformers, but is not included in the calculations explicitly.

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Conformational Energy Calculations

The nomenclature and conventions adapted by an IUPAC-IUB Commission were used throughout (2). Energy calculations were carried out with ECEPP (Empirical Conformational Energy Program for Peptides) (3). The empirical potential energy functions and energy parameters used are described elsewhere (4). Standard residue geometries were used as supplied in ECEPP and described elsewhere (4). Blocked end-groups (N-acetyl and amide) were chosen so that including the carba-bridge by models would be possible. Energy minimization was carried out using an algorithm described elsewhere (5). Minimization of the energy was terminated when the energy change from one cycle to the next was less than 0.01 Kcal/mol (41.8J). All dihedral angles, including peptide bonds (ω), were treated as variables in the minimization.

Starting conformations were chosen from a selected set of bend type conformations found from previous studies on peptapeptides (6,7), and from starting conformations denoted elsewhere (8). The conformations of the residue containing a D-configuration were chosen by inverting the ϕ,ψ values from dipeptide studies of L-isomers and the side-chain dihedral angles were adjusted appropriately (8,9). Extended conformations were not examined since only folded or bend type conformers could close the disulfide bridge. Different side-chain conformations were tested for each of the low-energy structures.

Results and Conclusions

The lowest energy structure of I is given in Table I, with the different combinations of the Phe and D-Trp side-chain positions. No other low-energy conformations were found. The bend occurs at the D-Trp-Lys positions, and is type II'. The position of the D-Trp ring in conformer A is such that the aromatic ring shields

TABLE I

LOW ENERGY CONFORMERS, ENERGIES, AND DISULFIDE GEOMETRY OF cyclo(Aha-Cys-Phe-D-Trp-Thr-Cys)

		Dihedral angle values, degrees			
Residue	Angle	A[a]	B[a]	C[a]	D[a]
Cys	ϕ	-145			
	ψ	135			
	ω	174			
	χ_1	-64			
Phe	ϕ	-84			
	ψ	87			
	ω	-173			
	χ_1	-59	-179	-58	179
	χ_2	107	62	107	63
D-Trp	ϕ	70			
	ψ	-134			
	ω	176			
	χ_1	180	-178	58	59
	χ_2	-89	-89	79	79
Lys	ϕ	-74			
	ψ	-37			
	ω	-178			
	χ_1	-66			
	χ_2b	-176(62)[c]			
Thr	ϕ	-72			
	ψ	99			
	ω	172			
	χ_1	36			
	χ_2	59			
	χ_3	60			
Cys	ϕ	-73			
	ψ	140			
	χ_1	-64			
Energy (Kcal/mol)		-12.2	-12.0	-9.3	-9.2
R_{SS} (Å)		2.039	2.045	2.039	2.043
χ_{SS} (Degrees)[d]		89.4	91.9	89.5	90.4

a) Dihedral angles in degrees. Those angles not listed are within ±4° of the angles of conformer A.

b) $\chi_{3,4,5} \approx \chi_2$.

c) χ_6 of Lys ($-NH_2$).

d) χ_{SS} is defined by the dihedral angle C_β-S-S-C_β.

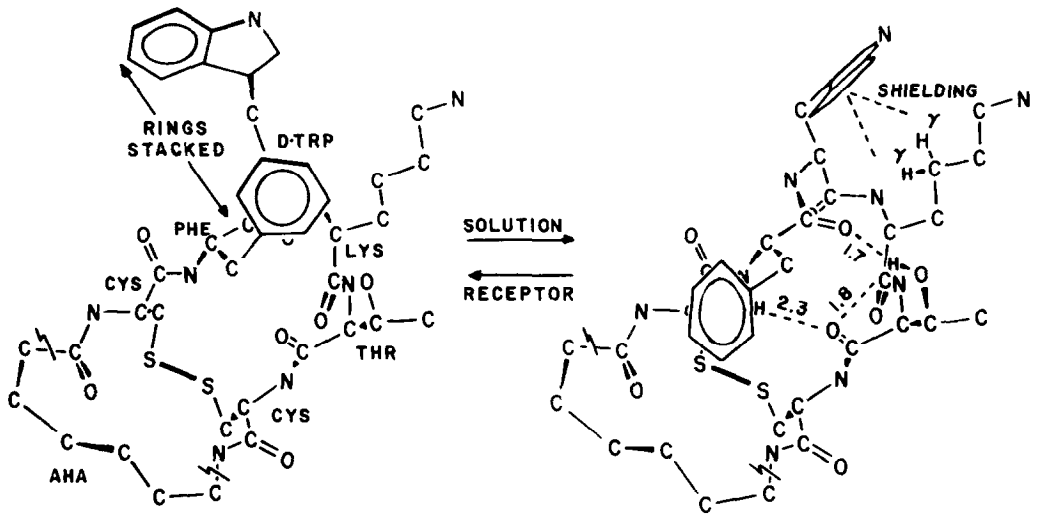

Figure 1. Solution and receptor conformations for cyclo(Aha-Cys-Phe-D-Trp-Lys-Thr-Cys). The carba-bridge was included by using models and stereochemical criterion.

the γ-hydrogens of the Lys side-chain (see Fig. 1). The disulfide ring is closed with a S-S distance of 2.039 Å and the dihedral angle about the S-S bond is +89°. Only little energy (∿0.2 Kcal) is lost upon moving the Phe side-chain from χ_1 = -59° to χ_1 = -179° (conformer B). Considerably more energy (∿3.0 Kcal) is lost upon moving the D-Trp side-chain from χ_1 = 180° to χ_1 = 58° (conformer C). Conformer D has the χ_1 of Phe at 179° and the χ_1 of D-Trp at +59°. This results in stacking the Phe-D-Trp rings one above the other at a ring to ring stacking distance of ∿6-7 Å (see Fig. 1). Structure D is ∿3.0 Kcal/mol higher in energy than A, with the energy loss coming from fewer close atom contacts. However, conformer D is in agreement with the ring stacking requirements found for a series of growth hormone agonist peptides (10,11,12), thus it is believed that a conformational change from conformer A to conformer D may occur upon binding to the cellular receptor. This change includes only side-chain reordering, but does not change the backbone conformation significantly. This induced conformational change would be

easily realized upon binding to the receptor, and brings this structure into excellent agreement with the binding mechanism proposed for the the pituitary growth hormone receptors (12). Conformer A, is most probably the solution structure, and is in agreement with a proposed model (13) as well as NMR data which shows that the γ-hydrogens of Lys are shifted upfield by the D-Trp ring. The temperature dependence of the NH resonances suggest that the Thr and Aha NH protons are buried or take part in hydrogen bonding (13). In the conformer proposed here the NH of Thr points toward the CO of Phe^3, and the NH of Aha points toward the CO of Cys^2. Further, the OH of Thr stabilizes this structure through hydrogen bonding to the CO of Phe ($H_{\cdots}O = 1.7$ Å). Since somatostatin (which has Phe^7-Trp^8) does not show the γ-hydrogen shift, but is the indogenous growth hormone release inhibitor, it would appear that the close Trp-Lys interaction described, is not required for the biologically active conformation. This also is in agreement with the receptor structure (conformer D) presented here. The carba-bridge was found to close easily with minimal variation in the end-group dihedral angles, and is included in Fig. 1.

A full discussion of the possible receptor binding and conformational transitions for both agonist and inhibitor activity at pituitary GRF receptors will be presented elsewhere (12).

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REFERENCES

1. Veber, D.F., Holly, F.W., Nutt, R.F., Bergstrand, S.J., Brady, S.F., Hirschmann, R., Glitzer, M.S., and Saperstein, R. (1979) Nature, 280, 512-514.

2. IUPAC-IUB Commission on Biochemical Nomenclature (1971) Biochem. J. 121, 577-585.

3. The Fortran program ECEEPP may be obtained from QCPE, Chemistry Chemistry Department, Rm 204, Indiana University, Bloomington, Indiana 47401. Order No. QCPE 286.

4. Momany, F.A., McGuire, R.F., Burgess, A.W. and Scheraga, H.A. (1975), J. Phys. Chem., 79, 2361-

5. Powell, M.J.D. (1964) Comput. J. 7, 155-162.

6. Momany, F.A. (1977) Biochem. Biophys. Res. Commun. 75, 1098-1103.

7. Isogai, Y. Nemethy, G. and Scheraga, H.A. (1977) Proc. Natl. Acad. Sci. USA, 74, 414-418.

8. Momany, F.A. (1976) J. Am. Chem. Soc. 98, 2990-2996.

9. Momany, F.A. (1978) J. Med. Chem. 21, 63-68.

10. Bowers, C.Y., Momany, F.A., Reynolds, G.A., Chang, D., Hong, A. and Chang, K (1980) Endocrinology, 106, 663-667.

11. Bowers, C.Y., Reynolds, G.A., Chang, D., Hong, A., Chang, K. and Momany, F.A., Accepted for publication, Endocrinology.

12. Momany, F.A., Bowers, C.Y., Hong, A. and Reynolds, G.A., Endocrinology, 1980 (Abstract).

13. Veber, D. (1979) Peptides, Proceedings of the 6th American Peptide Symposium, Gross, E. and Meienhofer, J. Eds.) Pierce Chemical Co., Rockford, Illinois, pp 409-419.