Biophysical chemistry of macrocycles for drug delivery: a theoretical study

*B. Khalili Hadad,[a] F. Mollaamin,[b] and M. Monajjemi[c,d]**

[a]*Department of Biological Sciences, Roudehen Branch, Islamic Azad University, Roudehen ,Iran*
[b]*Department of Chemistry, Qom Branch, Islamic Azad University, Qom,Iran*
E-mail: smollaamin@gmail.com
[c]*Department of Chemistry, Science and Research Branch, Islamic Azad University, Tehran, Iran*
[d]*Department of Chemistry and Biochemistry, Institute for Theoretical Chemistry, The University of Texas at Austin, Austin, Texas, USA.*
E-mail: m_monajjemi@cm.utexas.edu

Ab initio (RHF/STO-3G) quantum chemical calculations using the self-consistent reaction field (SCRF) model were carried out to analyze the effect of solvent polarity on the relative Gibbs free energies, the dipole moments, and the structural stability of peptide macrocycles based on unsubstituted cyclo[Gly_6] and its trisubstituted derivatives containing Me, NH_2, or OH groups at the Cα atom. The macrocycles studied are stable in water at both room temperature and at body fever temperature, which is important for the design of a stable nanovehicle for drug delivery in water.

Key words: peptide macrocycles, drug delivery, restricted Hartree—Fock approximation (RHF), self-consistent reaction field (SCRF) model.

Nanoparticles have been used as a tool for enhancement of the pharmacodynamic and pharmacokinetic properties of various drugs. They are used in an organism to control drug release in the circulation, to control access of the drug to specific sites, and to deliver the drug at a controlled rate to the action site. Various polymers, *e.g.*, biocompatible peptide systems, are used as nanoparticles for drug delivery research aimed at increasing the therapeutic benefit and minimizing side effects.[1—4]

Cyclic peptide molecules can be clustered by self-assembly to produce hollow peptide nanofibers. This can be treated as the spontaneous organization of individual components into an ordered structure.[5] The main point of molecular self-assembly is the complementary shape of the individual particles. In nature, principles of nano-fabrication engineering are mainly implemented through weak noncovalent interactions.[6—8]

Peptides, as a class of biological materials, have considerable potential for drug delivery.[9] Short peptides are easy to design and synthesize;[10] this makes them an ideal model system for studying biological self-assembly.

Some researches have shown that β-sheet peptide systems can undergo self-assembly. Also, fibers comprised of hollow peptide nanotubes have been designed to be inserted in bilayer lipids,[11] thus allowing ions to pass through.[12] Moreover, proteins have been synthesized that undergo self-assembly and form hydrogels sensitive to pH and some other environmental changes. A number of biomimetic peptide and protein structures combined with heme groups have also been studied.[13] Specific peptides forming complexes with metals and semiconducting elements have been investigated.[14] Although eixperimental research on the use of these systems has been carried out, they have not been considered as materials for drug delivery as yet. The use of peptides as biomaterials requires a thorough scientific understanding of all aspects of the interaction between drugs and peptide macrocycles. These efforts encouraged us to study nanomaterials containing biological constituents and the possibility to control their release properties.

In this work, we carried out quantum chemical calculations of the structures, dipole moments, and relative energies of some peptide macrocycles in the gas phase and in solvents of different polarity (water, DMSO, acetone, Et_2O) using the Hartree—Fock (RHF/STO-3G) method and the Onsager self-consistent reaction field (SCRF) model. The structural stabilities of the macrocycles with different substituents in different solvents were evaluated.

Calculations were performed in the temperature range from 290 K (somewhat lower than room temperature) to 315 K (fever temperature) and allowed us to locate the energetically stable backbone conformations of cyclo[Gly_6].[15,16]

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 2, pp. 233—236, February, 2011.
1066-5285/11/6002-238

Calculation Procedure

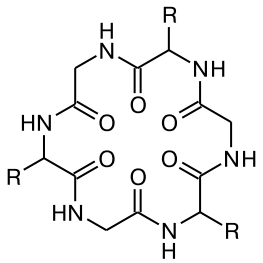
The calculations reported in this study were carried out with the Gaussian-98 program[17] and based on the geometries optimized by the RHF/STO-3G method. The stationary points located on the molecular potential energy hypersurfaces were characterized using standard analytical harmonic vibrational analysis.[18,19]

The effect of a solvent can be incorporated in quantum chemical calculations most easily by considering it as a continuous dielectric medium characterized by a dielectric constant. The electric field produced by the molecule induces a polarization of the medium, which in turn acts on the electrons in the molecule (SCRF). The model thus contains the quantum mechanical description of the molecule and a classical medium. In the Gaussian programs, a simple approximation was chosen, in which the volume of the solute is used to compute the radius of a cavity which forms the hypothetical surface of the molecule.[14,20]

Results and Discussion

The application of high-level quantum chemical calculations to more realistic systems is clearly desirable but exceeds the present computer capacity of most research groups. Considering the size of most bioinorganic systems, the Hartree—Fock methods therefore still seem to be the tool of choice in the description of relevant systems.

The original unsubstituted macrocycle was designed as a cyclic hexamer of glycine, cyclo[Gly_6] (**1**). In this study, we also have explored the ring systems with methyl (**2**), amino (**3**), and hydroxyl (**4**) groups in the side chain at C_α of the alternating glycine units.



1—4

R = H (**1**), Me (**2**), NH_2 (**3**), OH (**4**)

The effect of substituents on the dipole moment of the original ring in the gas phase and in different solvents is illustrated by the data of Table 1.

The results of calculations for the cyclic "peptide" **4** (see Table 1) demonstrate that in this case the variations of the dipole moment in different solvents were more evident than for peptides **1**, **2**, and "peptide" **3** and that the dipole moment itself showed a significant sensitivity to the solvent polarity. Surprisingly, all macrocycles considered exhibited a similar physicochemical behavior in water. Again this trend was more evident for the "peptide" **4**.

Table 1. Dipole moments (μ) of macrocycles **1**—**4** in the gas phase and in different solvents

Medium	μ/D			
	1	**2**	**3**	**4**
Gas phase	3.24	5.49	3.24	4.98
Water	3.65	6.11	4.27	9.23
DMSO	3.63	6.10	4.27	9.22
Acetone	3.63	6.07	4.25	9.17
Et_2O	3.52	5.91	4.14	8.90

In other words, the dipole moment of molecule **4** increases with an increase in the dielectric constant of the solvent and reaches a maximum value in water; this confirmed the higher stability of the macrocycles in water. These significant changes in the case of the cyclic "peptide" **4** can be due to the existing three hydroxyl groups. This considerably increases the possibility of formation of the network of intra- and intermolecular hydrogen bonds with water as a protic solvent.

In the case of cyclo[$(Gly\text{-}Ala)_3$] **2**, the solvent effect on the dipole moment values was insignificant. This can be related to the methyl groups that enhance the hydrophobic characteristics of the macrocycle.

The smallest values of the dipole moments in the gas phase were found for the cyclic peptide **1** and "peptide" **3** while the largest value was obtained for the "peptide" **4** in water. We expect that this ring may present the polar behavior in water. Generally, an increase in the calculated dipole moments is due to an increase in the dielectric constant of the solvent.

The solvent effects on the Gibbs free energies of macrocycles **1**—**4** at different temperatures are presented in Table 2.

The data of Table 2 show that the characteristics of peptide **2** with the methyl substituent at C_α vary only slightly depending on the solvent. From the point of view of the solvent bulk properties, these weak responses are fairly chaotic and, consequently, confirm the idea that the higher the temperature the higher ring stability should be observed. The most negative Gibbs free energy for compound **2** was obtained for water as solvent at 315 K. Water is the most proper solvent for this cyclic peptide at all temperatures studied. It is accepted that water is the best solvent in production of peptide nanotubes. One can expect that in water the cyclic molecule **2** will be more stable at both room temperature and body fever temperature, which is significant for the design of a stable drug delivery nanovehicle. Note also that water is more compatible with cells and body fluids. For comparison mention may be made that the stability of a peptide ring at the same temperature increases with an increase in the dielectric constant of the solvent.

Table 2. The effect of solvents on the Gibbs free energies of macrocycles at different temperatures

Temperature/K	$-\Delta G$/kcal mol^{-1}			
	1	**2**	**3**	**4**
		Water (ε = 77.39)		
290	0.00	0.00	0.00	0.00
	[768367.02]	[840910.02]	[870480.44]	[907238.05]
300	1.56	1.52	1.35	1.34
305	2.35	2.30	2.03	2.01
310	3.14	3.08	2.72	2.70
315	3.94	3.87	3.15	3.39]
		DMSO (ε = 46.80)		
290	0.00	0.00	0.00	0.00
	[768366.98]	[840910.00]	[870480.42]	[907238.00]
300	1.56	1.52	1.35	1.34
305	2.34	2.30	2.03	2.01
310	3.14	3.08	2.72	2.70
315	3.94	3.87	3.42	3.39
		Acetone (ε = 20.70)		
290	0.00	0.00	0.00	0.00
	[768366.98]	[840909.93]	[870480.39]	[907237.83]
300	1.56	1.52	1.35	1.34
305	2.35	2.30	2.03	2.01
310	3.14	3.08	2.72	2.70
315	3.94	3.87	3.42	3.39
		Et_2O (ε = 4.34)		
290	0.00	0.00	0.00	0.00
	[768366.76]	[840909.54]	[870481.52]	[907236.82]
300	1.56	1.52	1.37	1.34
305	2.34	2.30	0.68	2.01
310	2.34	3.08	1.37	2.70
315	3.94	3.86	2.07	3.39

Note. Numbers in square brackets represent the Gibbs free energies of macrocycles **1**—**4** in the corresponding solvent at 290 K. The relative Gibbs free energies (ΔG) at different temperatures are given relative to the Gibbs free energy at 290 K.

The highest stability of the cyclic "peptide" **3** containing amino groups at C_α was observed in DMSO at 315 K. A comparison of the results obtained for "compound" **3** at different temperatures and solvents shows that water is the best solvent for it. Although the stability of this system in water as solvent is lower than in Et_2O at 315 K, the negative Gibbs free energy is high enough to treat water as the proper solvent. Generally, the higher the dielectric constant of the solvent the higher the stability of the cyclic "peptide" **3**.

From the results obtained it follows that hydroxy-substituted cyclic "peptide" **4** is similar to compound **2**. The negative values of the Gibbs free energy increase as the temperature increases. A comparison of the data of Table 2 reveals similar structural stability with an increase in the dielectric constants of the solvent. From the data of Table 2 it also follows that water is the best solvent for "peptide" **4**.

Using the *ab initio* (RHF/STO-3G) method, in this study we unambiguously discriminated between different conformations of peptide macrocycles and concluded which conformation is more abundant in the gas phase and in solution.

It was shown that the dipole moments of unsubstituted cyclo[Gly_6] and its trisubstituted at C_α derivatives increase in the order H < NH_2 < OH < Me in the gas phase and in the order H < NH_2 < Me < OH in solvents of different polarity.

A comparison of the Gibbs free energy values for the cyclic peptides in different solvents showed that water is the best solvent for the peptide macrocycles studied. These rings is stable in water both at room temperature and at body fever temperature which is significant for the design of a stable drug delivery nanovehicle. In different solvents at the same temperature, the ring stability increases with increasing the dielectric constant of the solvent.

References

1. V. J. Mohanraj, Y. Chen, *Trop. J. Pharm. Res.*, 2006, **5**, 561.
2. M. C. Roco, *Curr. Opin. Biotechnol.*, 2003, **14**, 337.
3. A. Raval, A. Choubey, C. Engineer, H. Kotadia, D. Kothwala, *Trends Biomater. Artif. Organs*, 2007, **20**, 101.
4. *Biomedical Nanotechnology*, Ed. N. H. Malsch, CRC Press, Boca Raton, 2005, 232 pp.
5. G. M. Whitesides, M. Boncheva, *Proc. Natl. Acad. Sci. USA*, 2002, **99**, 4769.
6. Sh. Zhang, D. M. Marini, W. Hwang, S. Santoso, *Curr. Opin. Chem. Biol.*, 2002, **6**, 865.
7. M. Monajjemi, V. S. Lee, M. Khaleghian, B. Honarparvar, F. Mollaamin, *J. Phys. Chem. C*, 2010, **114**, 15315.
8. M. Monajjemi, L. Mahdavian, F. Mollaamin, *Bull. Chem. Soc. Ethiop.*, 2008, **22**, 227.
9. S. Zhang, in *Encyclopedia of Materials Science and Technology*, Elsevier, Oxford, 2001, p. 5822.
10. W. C. Chan, P. D. White, *Fmoc Solid Phase Peptide Synthesis: A Practical Approach*, Oxford University Press, New York, 2000, p. 41.
11. A. Aggeli, I. A. Nyrkova, M. Bell, R. Harding, L. Carrick, T. C. B. McLeish, A. N. Semenov, N. Boden, *Proc. Natl. Acad. Sci. USA*, 2001, **98**, 11857.
12. M. Monajjemi, L. Mahdavian, F Mollaamin, M. Khaleghian, *Zh. Neorg. Khim.*, 2009, 1536 [*Russ. J. Inorg. Chem.* (*Engl. Transl.*), 2009, **54**, 1465].
13. W. A. Petka, J. L. Harden, K. P. McGrath, D. Wirtz, D. A. Tirrell, *Science*, 1998, **281**, 389.
14. S. R. Whaley, D. S. English, E. L. Hu, P. F. Barbara, A. M. Belcher, *Nature*, 2000, **405**, 665.
15. N. Mora-Diez, M. L. Senent, B. Garcia, *Chem. Phys.*, 2006, **324**, 350.
16. D. C. Young, *Computational Chemistry: A Practical Guide for Applying Techniques to Real-World Problems*, John Wiley and Sons, New York—Weiheim, 2001, 381 pp.

17. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, V. G. Zakrzewski, J. A. Montgomery, R. E. Stratmann, J. C. Burant, S. Dapprich, J. M. Millam, A. D. Daniels, K. N. Kudin, M. C. Strain, O. Farkas, J. Tomasi, V. Barone, M. Cossi, R. I. Camm, B. Mennucci, C. Pomelli, C. Adamo, S. Clifford, J. Ochterski, G. A. Petersson, P. Y. Ayala, Q. Cui, K. Morokuma, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Raghavachari, J. Cioslowski, J. V. Ortiz, A. G. Baboul, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. Gomperts, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, C. Gonzalez, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, J. L. Andres, C. Gonzalez, M. Head-Gordon, E. S. Replogle, J. A. Pople, *Caussian-98, Revision A.7*, Gaussian, Inc., Pittsburgh (PA), 1998.
18. S. Sairam, S. Mallaj, D. Ayan, K. Pati Swapan, *Synth. Metals*, 2005, **155**, 398.
19. A. E. Reed, L. A. Curtiss, F. Weinhold, *Chem. Rev*., 1988, **88**, 899.
20. M. Witanowski, Z. Biedrzycka, W. Sicinska, Z. Grabowski, *J. Molec. Structure*, 2002, **602—603**, 199.

Received January 1, 2009;
in revised form June 9; 2010