Biphenyl Twist Sense Linkage between Side-Chain and Main-Chain Conformations in a Polyglutamate Ester

MASAHIKO SISIDO,*,† MICHAEL P. REIDY,‡ AND
MARK M. GREEN*,‡

Research Laboratory of Resources Utilization, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama 227, Japan, and Department of Chemistry and Polymer Research Institute, Polytechnic University, Six MetroTech Center, Brooklyn, New York 11201

Received June 5, 1991;
Revised Manuscript Received September 9, 1991

Introduction

Since the structure and conformation of the side chains of the amino acid residues in proteins overwhelmingly control the higher order structures of these macromolecules,[1,2] much work has been directed both to the theoretical calculation of the side-chain conformation as related to the main-chain conformation in polypeptides and to the experimental test of these calculations.[3]

One opportunity to test the correlation of theory and experiment in the side-chain conformations of polypeptides arises from the observation that the biphenyl chromophore in the side chain of poly[γ-(*p*-phenylbenzyl) L-glutamate] (PPBLG) gives rise, in dilute solution in several solvents, to a substantial circular dichroism (CD) which is lost on denaturation of the α-helical backbone.[2] This result requires that the local mirror image twist senses about the biphenyl bond are substantially unequal in energy in spite of the 10 bonds intervening between the coupled chiral units, i.e., the biphenyl group and the α helix. Since this coupling must depend on the side-chain conformation and since the extensive work on optically active small-molecule bridged biphenyls[4] allows translation of the CD behavior of PPBLG into the twist sense preference, i.e., left-handed, and with less certainty, the approximate excess of this sense of between 12 and 24%, the observation[2] offers an opportunity to use an unusually subtle parameter to compare experiment to theory in the area of force-field calculations of the polypeptide side-chain conformation.[3,5]

In the work presented in this paper, we applied a polypeptide force field to the side chain of PPBLG in the right-handed α-helical form and discovered that the most populated side-chain conformations exhibit a preference for a left-handed twist about the biphenyl bond.

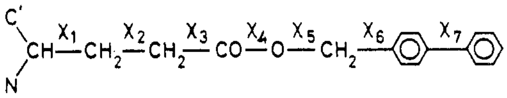
Force-Field Calculations

The potential energy and structural parameters are taken from the ECEPP system.[6] The biphenyl group structure was input from X-ray crystallographic data.[7] The crystal data show a planar structure, but in solution the two rings are twisted by close to 40°.[8] The partial charges of the biphenyl group were calculated using CNDO/ON molecular orbital theory using the model compound biphenylmethyl *N*-acetyl-L-glutamate *N*-methylamide with an extended side-chain orientation (χ_1 to χ_6 equals 180°).[9] The rotational potentials around χ_1 through χ_6 (Table I) are taken from the literature.[10] In this literature,[10] the potential function inherent to rotation around the C^γ–C^δ(=O) bond (χ_3) is neglected [$V(\chi_3) = 0$], due to the approximate 6-fold symmetry. This choice of potential resulted in the energy of the gauche state ($\chi_3 = 60°$) to be lower by about 0.2 kcal/mol than the trans state (C^β–C^γ bond eclipsing the C=O bond, $\chi_3 = 180°$) in methyl γ-biphenylmethyl *N*-acetyl-L-glutamate, due to van der Waals and electrostatic interactions in other parts of the side chain. However, Siam et al.[11] reported that a trans state is more stable than a gauche state by about 1 kcal/mol in propanoic acid and suggested a potential function: $V(\chi_3) = 0.485(1 + \cos\chi_3) + 0.0305(1 - \cos 2\chi_3) + 0.555(1 + \cos 3\chi_3)$. We carried out two series of computations. First, the rotational potential was neglected as in the literature,[10] and, second, the potential function of Siam et al.[11] was used.

Table I
Low-Energy Conformations of Poly(biphenylmethyl L-glutamate) (n = 20) in α-Helix ($\phi = -62.5°$, $\psi = -42.3°$, $\omega = 180°$)[a]

χ_1	χ_2	χ_3	χ_4	χ_5	χ_6	χ_7	V, kcal/residue
a. PPBLG [$V(\chi_3) = 0$]							
191	65	231	180	179	11	−40	−14.56
287	167	98	180	66	34	−40	−14.20
185	179	34	180	181	77	40	−13.66
188	61	220	180	179	159	−40	−13.33
187	177	38	180	181	118	−40	−12.95
185	177	152	180	65	55	40	−12.79
190	185	317	180	181	54	40	−12.57
186	63	219	180	177	58	40	−12.41
186	176	179	180	179	148	−40	−6.47
186	176	179	180	179	148	40	−6.46
b. PPBLG [$V(\chi_3) = 0.485(1 + \cos\chi_3) + 0.305(1 - \cos 2\chi_3) + 0.555(1 + \cos 3\chi_3)$]							
191	64	228	180	181	10	−40	−9.10
188	177	35	180	178	77	40	−8.54
184	177	155	180	64	57	40	−8.44
186	60	217	180	179	160	−40	−8.39
288	167	96	180	67	32	−40	−8.35
290	160	195	180	181	−1	−40	−8.04
188	176	38	180	181	121	−40	−7.84
184	62	213	180	181	69	40	−7.72
291	156	193	180	180	153	40	−7.60
191	184	316	180	181	52	40	−7.49
288	168	199	180	181	148	−40	−7.28
172	61	206	180	180	118	40	−7.25
186	176	179	180	179	148	−40	−2.88
186	176	179	180	179	148	40	−2.87
c. PBLG [$V(\chi_3) = 0$]							
186	63	238	180	181	4		−12.44
285	180	172	180	294	126		−11.06
286	170	91	180	67	33		−10.58

[a] Energy minimization was carried out for χ_1, χ_2, χ_3, χ_5, and χ_6. χ_4 is fixed to 180°. χ_7 was set to ±40°. All-trans conformations with $\chi_7 = \pm 40°$ are also included. Low-energy side-chain conformations of PBLG (n = 20) in α-helix are also listed.



Calculations were carried out on an NEC PC 9801 computer equipped with a numerical data processor and coded with MS-Fortran. The main chain was assigned the right-handed α-helical conformation with $\phi = -62.5°$, $\psi = -42.3°$, and $\omega = 180°$.[12] An initial trial using a g^-tg^+ rotational isomeric state (RIS) model was applied to the side-chain units in a 20-mer of the biphenyl L-glutamate, and later in the energy minimization all angles were allowed to take arbitrary values as discussed below. At all levels in the calculation the 20 side-chain units were assigned to the same conformation although the various angles in each side chain were allowed to change independently to a

---

† Tokyo Institute of Technology.
‡ Polytechnic University.

0024-9297/91/2224-6860$02.50/0

common energy minimum (see the Results and Discussion section). The initial rotational angles of each bond are taken from published data.[13] Following the literature in this area,[3,10,13] the ester bond was fixed to a trans conformation, i.e., $\chi_4 = 180°$. Similarly,[3,10] χ_6 was initially evaluated from 0 to 180° at 30° intervals. The twisting angle of the biphenyl group χ_7 was allowed only two states, +40° (right handed) and −40° (left handed).

First, calculations were made assuming $V(\chi_3) = 0$. Potential energies were calculated for the 972 rotational isomeric states, resulting in 160 states with energies within 8.25 kcal/mol residue. Starting from the 160 conformations, energy minimizations were performed, resulting in 107 different minimum energy conformations. The eight side-chain conformational states within the 2.15 kcal/mol residue, which would therefore comprise greater than 97% of the population at room temperature, are listed in Table I. Also included are two higher energy all-trans states differing in χ_7. The procedures described above were also applied to poly(γ-benzyl L-glutamate) (PBLG), and the three most stable side-chain conformational states are also exhibited in Table I (see the Results and Discussion section). The same procedures were then carried out using $V(\chi_3)$ from the literature.[11] The results are also listed in Table I.

Results and Discussion

The three most populated side-chain conformations of PPBLG in Table Ia [$V(\chi_3) = 0$] are exhibited[14] as ball and stick models in Figure 1a–c and demonstrate the strong preference, predicted by the force field, for the side chain to occupy space adjacent to the helical main chain. A population preference of about 60% for the left-handed twist sense about the biphenyl bond can be calculated from the relative energies in Table I and the values of χ_7.

The detailed responsible factors for the biphenyl twist preference were evaluated by studying the energy in each conformational state in Table I as a function of the number of residues and the biphenyl twist sense. Thus, in the lowest energy conformation (Table Ia) we find no energy differences between the biphenyl twist senses until the eighth residue is added. This causes a high-energy contact, when the biphenyl is right handed, between the meta hydrogen of the terminal phenyl ring in residue one with the carbonyl oxygen of residue eight. In order to adapt to the unfavorable twist sense, the force field finds another minimum by moving χ_6 ($E = -12.41$, Table Ia). Similarly, we find that nonproximal side-chain interactions are responsible for the biphenyl twist sense differences found in the other states shown in Table Ia.

The absence of input from solvation, the assumption of locked biphenyl and ester geometries at 40 and 180°, and the necessary computational expediency of allowing no disorder in the side-chain structure[10] causes one to pause in taking the extreme details literally. Nevertheless, indifference to the biphenyl twist sense of the high-energy all-trans conformations in Table Ia supports the intuitively reasonable conclusion that proximity of the biphenyl group and the helix is necessary for the distinction observed experimentally.[2] The force field shows us that the crowding following from this proximity then causes the side-chain interactions which directly control the biphenyl twist sense. The intensity of the CD signal demonstrates a less than quantitative preference for the left-handed twist state about the biphenyl group,[2] and this is also consistent with the force field which shows several side-chain conformations with similar energies and with varying preferences for the biphenyl twist sense (Table Ia).

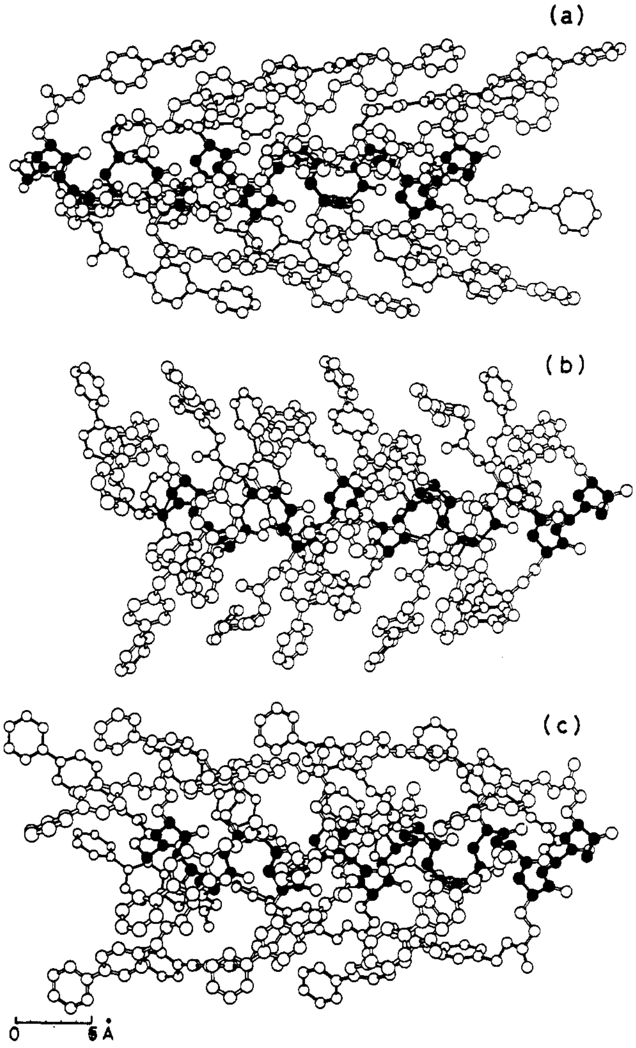


Figure 1. Ball and stick molecular models for the three most populated side-chain conformations of PPBLG obtained with $V(\chi_3) = 0$ (Table Ia). The conformation with the lowest energy is a, the second is b, and the third is c. A NAMOD molecular display routine[14] was used.

In order to evaluate if the above conclusion is dependent on the choice of potential functions, especially for χ_3 where controversy exists, similar calculations using the rotational potential of Siam et al.[11] were carried out. The results are listed in Table Ib. Although their order was exchanged, the low-energy conformations found in the former calculation appeared in the latter case. Particularly, the most populated conformations are the same in both cases. Therefore, under the restriction of trans ester linkage, the side chain is still predicted to be folded, causing one of the enantiomeric forms of the biphenyl group to be more stable, as in Table Ia.

Although the nonproximal side-chain interactions predicted by the force field to be necessary for the observations[2] require the same side-chain conformations for at least small distances, i.e., two or three turns along the polypeptide, our results offer no answer to the question[13] of the distribution of the side chains or among chains and the details of such distributions.[15] Meticulous dielectric dispersion experiments[16] demonstrate a contribution to the overall dipole moment from the side chains in poly(γ-benzyl L-glutamate) suggesting extensive order in agreement with the CD observations[2] and with the calculations reported here and previous calculations on PBLG.[10]

The results in Table I for PBLG show similar low-energy conformations to PPBLG. This suggests that our conclusion, based on the correlation of the experimental and theoretical work discussed above, in general agreement with the original calculations of Scheraga and his coworkers,[10] should be encountered in other polyglutamates. However, these states may be mixed with other side-chain states in unknown ways which might, considering the small energy differences among the side-chain conformations, depend on the state of the polymer sample.[17]

Acknowledgment. We are grateful to the donors of the Petroleum Research Fund, administered by the American Chemical Society, to the National Science Foundation (Grant CHE-8615872), and to IBM Corp. (Almaden Laboratory) for financial support for this work, which was conducted with the help of a U.S.–Japan Fellowship to M.M.G. We are indebted to Professor Akihiro Abe of the Tokyo Institute of Technology for helpful discussions on the computations.

References and Notes

(1) Fasman, G. D. *Poly-α-Amino Acids*; Marcel Dekker: New York, 1967. Fasman, G. D. *Biopolymers* **1987**, *26*, S59. Liff, M. I.; Lyu, P. C.; Kallenbach, N. R. *J. Am. Chem. Soc.* **1991**, *113*, 1014. Lyn, P. C.; Wang, P. C.; Liff, M. I.; Kallenbach, N. R. *J. Am. Chem. Soc.* **1991**, *113*, 3568.

(2) Reidy, M. P.; Green, M. M. *Macromolecules* **1990**, *23*, 4225.

(3) Piela, L.; Nemethy, G.; Scheraga, H. A. *Biopolymers* **1987**, *26*, 1273.

(4) Mislow, K.; Glass, M. A. W.; O'Brien, R. E.; Rutkin, P.; Steinberg, D. H.; Weiss, J.; Djerassi, C. *J. Am. Chem. Soc.* **1962**, *84*, 1455. Bunnenberg, A.; Djerassi, C.; Mislow, K.; Moscowitz, A. *J. Am. Chem. Soc.* **1962**, *84*, 2823, 5003. Mislow, K.; Bunnenberg, E.; Records, R.; Wellman, K.; Djerassi, C. *J. Am. Chem. Soc.* **1963**, *85*, 1342.

(5) Sisido, M.; Imanishi, Y. *Macromolecules* **1986**, *19*, 2187. Sisido, M. *Macromolecules* **1989**, *22*, 3280. Sisido, M. *Macromolecules* **1989**, *22*, 4367. Sisido, M.; Okamoto, A.; Imanishi, Y. *Polym. J.* **1985**, *17*, 1263.

(6) Momany, F. A.; McGuire, R. F.; Burgess, A. W.; Scheraga, H. A. *J. Phys. Chem.* **1975**, *79*, 2361.

(7) Robertson, G. B. *Nature* **1961**, *191*, 593.

(8) Barrett, R. M.; Steele, D. *J. Mol. Struct.* **1972**, *11*, 105. Grinter, R. *Mol. Phys.* **1966**, *11*, 7. Unanue, A.; Bothorel, P. *Bull. Soc. Chem. Fr.* **1966**, 1640.

(9) We are indebted to Professor H. A. Scheraga of Cornell University for supplying the CNDO/ON program. The list of partial charges is available on request from M.S.

(10) Ooi, T.; Scott, R. A.; Vanderkooi, G.; Scheraga, H. A. *J. Chem. Phys.* **1967**, *46*, 4410. Yan, J. F.; Vanderkooi, G.; Scheraga, H. A. *J. Chem. Phys.* **1968**, *49*, 2713.

(11) Siam, K.; Klimkowski, V. J.; Ewbank, J. D.; Schäfer, L. *J. Comput. Chem.* **1984**, *5*, 451.

(12) Blundell, T.; Barlow, D.; Borkakoti, N.; Thornton, J. *Nature* **1983**, *306*, 281.

(13) Abe, A.; Yamazaki, T. *Macromolecules* **1989**, *22*, 2138.

(14) Beppu, Y. *Comput. Chem.* **1989**, *13*, 101.

(15) This is a longstanding question in this field. See ref 10 and also: Chen, A. K.; Woody, R. W. *J. Am. Chem. Soc.* **1971**, *93*, 29.

(16) Nishioka, N.; Mishima, H.; Teramoto, A. *Polym. J.* **1977**, *9*, 641. See also: Erenrich, E. H.; Scheraga, H. A. *Macromolecules* **1972**, *5*, 746.

(17) Compare for example the results on solid-state measurements in: Meirovitch, E.; Samulski, E. T.; Leed, A.; Scheraga, H. A.; Rananavare, B.; Nemethy, G.; Freed, J. H. *J. Phys. Chem.* **1987**, *91*, 4840. Romero Colomer, F.; Gomez Ribelles, J. L. *Polymer* **1989**, *30*, 849.