A New Set of Molecular Mechanics Parameters for Hydroxyproline and Its Use in Molecular Dynamics Simulations of Collagen-Like Peptides

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Abstract: Recently, the importance of proline ring pucker conformations in collagen has been suggested in the context of hydroxylation of prolines. The previous molecular mechanics parameters for hydroxyproline, however, do not reproduce the correct pucker preference. We have developed a new set of parameters that reproduces the correct pucker preference. Our molecular dynamics simulations of proline and hydroxyproline monomers as well as collagen-like peptides, using the new parameters, support the theory that the role of hydroxylation in collagen is to stabilize the triple helix by adjusting to the right pucker conformation (and thus the right ϕ angle) in the Y position.

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Introduction

One of the unique features of collagen is the hydroxylation of prolines.[1,2] The structure of collagen is a triple helix composed of three chains wrapped around each other, and each chain is made of repetition of Xaa-Yaa-Gly triplets, where the X and Y positions are often occupied by prolines. Hydroxylation of prolines occurs post-translationally, further stabilizing the triple helix. Intriguingly, only those prolines in the Y position are hydroxylated *in vivo*, and *in vitro* hydroxylation of X-position prolines destabilizes the triple helix.[3] This selectivity in particular has stimulated the quest for the role of hydroxyprolines in collagen. (Another interesting question regarding the hydroxylation in collagen is how prolyl hydroxylase recognizes prolines in the Y position; there is some evidence that the β-bend conformation plays an important role.[4])

It was first suggested that the hydroxyl group of hydroxyproline forms hydrogen bonds with surrounding water molecules, thereby stabilizing the collagen triple helix in water.[5,6] This suggestion, however, does not explain the fact that only the Y-position hydroxyprolines stabilize the triple helix. Besides, collagen-like peptides made of Pro-Flp-Gly triplets, where prolines in the Y position are replaced with fluoroprolines instead of hydroxyprolines, are known to be even more stable than those made of Pro-Hyp-Gly triplets despite the inability of fluorine to make hydrogen bonds.[7]

Recently, the ring pucker conformation has been proposed as a reason for the hydroxylation.[8] There are two different conformations of the ring pucker (Fig. 1): in the C^{γ}-endo conformation the C^{γ} atom is placed towards the C^{α}—C bond, and in the C^{γ}-exo conformation it is placed away from the C^{α}—C bond. There exists a close correlation between the pucker and the ϕ angle due to the ring constraint. Therefore, the theory is that proline prefers the endo pucker, which leads to the ϕ angle that is optimal for the X position, whereas hydroxyproline prefers the exo pucker, which leads to the ϕ angle that is optimal for the Y position. This theory is supported by high-resolution crystal structures of collagen-like peptides showing clear distinctions in the pucker and the ϕ angle between the X and Y positions.[8,9]

Computational methods have been in use for the study of collagen,[11–14] complementing experimental methods. However, as shown later in this article, the previously developed molecular mechanics parameters for hydroxyproline[11,13] are not entirely satisfactory in reproducing correct pucker preferences. Considering

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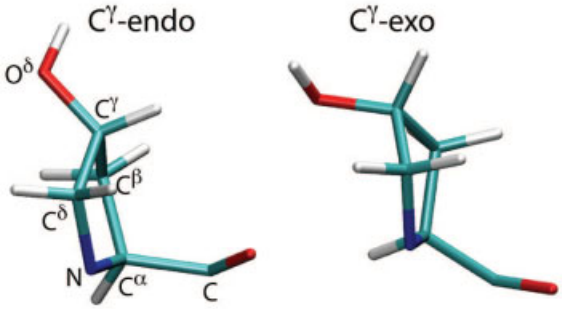

Figure 1. Two different ring pucker conformations of Pro/Hyp. The figure is drawn for hydroxyproline. Made with VMD.[10] [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

the presumed importance of the pucker in the stability of collagen, improvement of the hydroxyproline parameters is urgently needed.

Here we develop new molecular mechanics parameters for hydroxyproline based on quantum mechanical calculations, paying particular attention to the endo/exo pucker conformations. (For another parameterization effort that takes into account Pro/Hyp ring pucker conformations, see ref. 15.) We use the new parameters in molecular dynamics simulations of Pro/Hyp monomers and collagen-like peptides, and find that (1) when placed in the X position, proline retains the endo pucker conformation, which is its natural conformation seen in the monomer, but when placed in the Y position, its pucker is shifted towards the exo; and (2) hydroxyproline in the Y position retains the exo pucker conformation, which is its natural conformation seen in the monomer. This is consistent with the aforementioned theory explaining why prolines only in the Y position need to be hydroxylated.

Parameterization of Hydroxyproline

The exo pucker preference of hydroxyproline is believed to be caused by a *gauche* effect.[16,17] In the endo pucker conformation, the N–C^{δ}–C^{γ}–O^{δ} dihedral is placed near the *anti* conformation; in the exo pucker conformation, on the other hand, it adopts a *gauche* conformation. The electron-withdrawing ability of the hydroxyl group stabilizes the *gauche* conformation, and therefore the exo pucker. It is thus most reasonable to focus on the N–C^{δ}–C^{γ}–O^{δ} torsion energy in the parameterization of hydroxyproline.

A set of molecular mechanics parameters for hydroxyproline was developed previously,[11,13] based on the AMBER 94 force field.[18] It consists of point charges that were obtained with the restrained electrostatic potential methodology[19] and a torsion energy for the N–C^{δ}–C^{γ}–O^{δ} dihedral that was parameterized using the N-β-ethyl amide model. All the other parameters are taken from the default values in the AMBER 94 force field according to atom types. This set of parameters, however, does not correctly reproduce the pucker preference of hydroxyproline (Section 3).

We have developed a new set of parameters for hydroxyproline based on the AMBER 99 force field.[20] (Thus, the new parameters are to be used with the AMBER 99 force field. The upgrade from AMBER 94 to 99, however, is not the main improvement here; it is the new parameterization of the N–C^{δ}–C^{γ}–O^{δ} torsion that leads to the reproduction of correct pucker preferences.) We kept the previously developed point charges, reproduced here in Table 1 (after all, point charges of standard residues were not changed from AMBER 94 to 99), but parameterized the N–C^{δ}–C^{γ}–O^{δ} torsion in a different way. Instead of using a simple molecule such as N-β-ethyl amide, we used hydroxyproline itself; the N–C^{δ}–C^{γ}–O^{δ} torsion energy was tweaked until the energy difference between the endo and exo pucker conformations matched the outcome of a quantum mechanical calculation. (Fitting gas-phase quantum mechanical energies with a molecular mechanics charge model designed to reproduce aqueous charge distributions is not quite consistent; there have been attempts to address this issue.[21]) The detailed procedure and the result of the parameterization follows.

Quantum Mechanical Calculation

We have calculated the energy difference between the endo and exo pucker conformations of hydroxyproline through a quantum mechanical calculation using Gaussian 98.[22] Two conformations of hydroxyproline monomer (capped with acetyl and NH_2 end groups) were prepared, one with the endo pucker and the other with the exo pucker. Each conformation was energy-minimized using the 6-31G* basis set at the Hartree–Fock level. Because the ψ angle is rather flexible, it was constrained at 152°, the typical value for hydroxyprolines in collagen,[23] during the energy minimization. Therefore, care must be taken if the present parameters are to be used in a noncollagen context. For comparison, the same procedure was applied to proline as well, except that the ψ angle was constrained at 163°, which is the typical value for proline in the X position.[23]

The results of the quantum mechanical calculations are summarized in Table 2. An easy indicator of pucker conformation is the χ_1 dihedral angle (N–C^{α}–C^{β}–O^{γ}); it is positive for the endo pucker and negative for the exo pucker, and there is clear separation between them.[8,9] The dihedral N–C^{δ}–C^{γ}–O^{δ}, which we focus on for the parameterization of hydroxyproline, is labeled ξ. For proline, we define ξ to denote N–C^{δ}–C^{γ}–H^{γ}, where H^{γ} is the hydrogen atom that is replaced with the hydroxyl group in the hydroxylation.

The endo conformation of proline has a similar ring structure as the endo conformation of hydroxyproline, as can be seen from the values of the three dihedrals angles, ϕ, χ_1, and ξ; the same applies

Table 1. Point Charges for Hydroxyproline.[a]

Atom	Charge	Atom	Charge	Atom	Charge
N	−0.2548	HG	0.0416	HB2	0.0426
CD	0.0595	OD	−0.6134	CA	0.0047
HD1	0.0700	HO	0.3851	HA	0.0770
HD2	0.0700	CB	0.0203	C	0.5896
CG	0.0400	HB1	0.0426	O	−0.5748

[a]Developed in ref. 11.

Table 2. Quantum Mechanical Energy Minimization.[a,b,c]

	Pucker	ϕ	χ_1	ξ	ψ	Energy[d]
Pro	endo	−72.1	33.7	147.9	163.0	0
	exo	−59.4	−21.0	81.7	163.0	0.94 kcal/mol
Hyp	endo	−70.1	31.8	147.4	152.0	0
	exo	−60.1	−27.6	79.5	152.0	−0.48 kcal/mol

[a]Angles are in degrees.
[b]ψ angles were constrained at 163° for Pro and at 152° for Hyp.
[c]For Hyp, ξ is the dihedral N—C^{δ}—C^{γ}—O^{δ}. For Pro, it is N—C^{δ}—C^{γ}—H^{γ}, where H^{γ} is the hydrogen atom that is replaced with the hydroxyl group in the hydroxylation.
[d]Shown is the minimized energy of the exo pucker conformation relative to the respective endo pucker conformation.

to the exo conformations as well. But, for proline the endo conformation has a lower energy than the exo, while hydroxyproline shows the opposite tendency. It is also noticeable that the ϕ angles of the minimized structures coincide with the average values known for the collagen triple helix. (The average ϕ angle is about −74° for the X position and about −60° for the Y position.[23]) Considering the energy differences and the ϕ angles together, our quantum mechanical calculation implies that proline is energetically preferred in the X position and hydroxyproline is energetically preferred in the Y position.

Molecular Mechanics Parameterization

With the energy difference $\Delta E = E^{\text{exo}} - E^{\text{endo}} = -0.48$ kcal/mol (obtained from the quantum mechanical calculation) in hand, we parameterized the ξ dihedral angle (N–C^{δ}–C^{γ}–O^{δ}) based on the AMBER 99 force field[20] and the previously determined point charges.[11] Without any special parameterization, the torsion energy of the ξ angle would be chosen by the AMBER 99 force field according to atom types: it would be $0.156\,(1 + \cos 3\xi)$ kcal/mol, which is a general formula for any X–CT–CT–X dihedral angles. This formula, however, is not sufficient to capture the *gauche* effect; in fact, with this formula alone, the *anti* conformation would be preferred to the *gauche* conformation. Therefore, as in ref. 13, we added another term $A\,(1 + \cos 2\xi)$, where the amplitude A represents the magnitude of the *gauche* effect.

Starting with the initial value $A_0 = 0$, the amplitude A was determined through the following iterative procedure: (1) Given A_n at the nth iteration step, energy-minimize endo and exo conformations of the hydroxyproline monomer (capped with acetyl and NH_2 end groups). (2) Measure the ξ angles, ξ_n^{endo} and ξ_n^{exo}, and the energy difference, $\Delta E_n = E_n^{\text{exo}} - E_n^{\text{endo}}$, of the energy-minimized conformations. (3) Update the amplitude A by

$$A_{n+1} = A_n + (\Delta E - \Delta E_n)/(\cos 2\xi_n^{\text{exo}} - \cos 2\xi_n^{\text{endo}}).$$

This procedure was iterated until ΔE_n reached the target value $\Delta E = -0.48$ kcal/mol. Even though an update of A according to the above equation exactly compensates the discrepancy between ΔE_n and ΔE, it also changes the ξ angles of the energy-minimized conformations; that is why iteration is needed. The energy minimization was done with AMBER 8 molecular simulation package,[24] with the ψ angle constrained at 152° as in the quantum mechanical calculation.

After three iteration steps, the target ΔE was reached and the final value of 1.49 kcal/mol was obtained for the amplitude A. Therefore, we propose

$$[0.156(1 + \cos 3\xi) + 1.49(1 + \cos 2\xi)] \quad \text{kcal/mol}$$

for the torsion energy of the N–C^{δ}–C^{γ}–O^{δ} dihedral. The measurements of the ϕ, χ_1, ξ, and ψ dihedral angles for the final endo and exo conformations of hydroxyproline are listed in Table 3. For comparison, these angles and energies were also measured for the endo and exo conformations of the proline monomer (capped with acetyl and NH_2 end groups) energy-minimized with the AMBER 99 force field (we have not done any reparameterization of proline). Compared with our quantum mechanical result, the AMBER 99 force field, at least qualitatively, captures the energetic preference of the endo conformation to the exo conformation of proline. The discrepancy in the actual values (1.44 vs. 0.94 kcal/mol) is not surprising at all, because proline was not parameterized against this energy difference.

Molecular Dynamics Simulation of Pro/Hyp Monomers

Our parameterization of hydroxyproline was done in such a way that the difference of the minimized energies of the endo and exo conformations matches the value calculated from a quantum mechanical calculation. It is, however, a different issue how this new set of parameters will affect molecular dynamics (MD) simulations at nonzero temperature. We have performed MD simulations of proline and hydroxyproline monomers at 300 K to observe the pucker preference in each residue.

A proline monomer and a hydroxyproline monomer, capped with the acetyl and NH_2 end groups, were each simulated for 10 ns at a constant temperature of 300 K using the AMBER 8 molecular simulation package.[24] Temperature was controlled using the Langevin dynamics with a collision frequency of 0.02 ps^{-1}, and the generalized Born method[25,26] was used as an implicit solvent model. A time step of 2 fs was used, with all bond lengths constrained with the SHAKE algorithm.[27]

Table 3. Molecular Mechanical Energy Minimization.[a,b]

	Pucker	ϕ	χ_1	ξ	ψ	Energy[d]
Pro	endo	−74.2	31.7	145.8	163.0	0
	exo	−58.9	−20.7	84.2	163.0	1.44 kcal/mol
Hyp	endo	−74.5	29.7	135.8	152.0	0
	exo	−56.8	−22.7	88.0	152.0	−0.48 kcal/mol

[a]See the caption of Table 2 for the definitions of the angles.
[b]The AMBER 99 force field was used with the new hydroxyproline parameters.

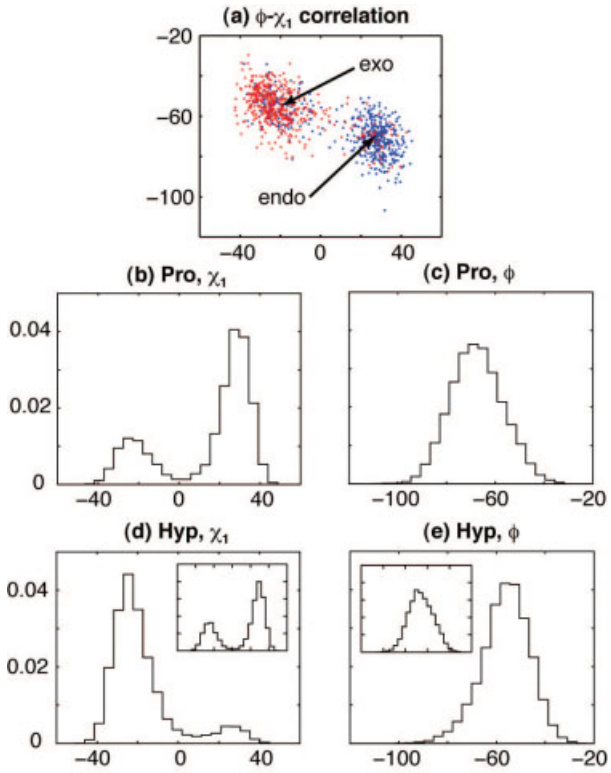

Figure 2. MD simulations of Pro/Hyp monomers. (a) Correlation between ϕ and χ_1 values sampled from the trajectories of proline (blue) and hydroxyproline (red). (b)–(e) Normalized histograms of ϕ and χ_1. Insets in (d) and (e) were obtained with the previous hydroxyproline parameters.[13]

From each MD trajectory, the ϕ and χ_1 angles were recorded every picosecond. On the ϕ–χ_1 plot in Figure 2a, the endo and exo pucker conformations appear as two distinct clusters; the center of the endo cluster is located around (25°, −70°) and that of the exo cluster is located around (−25°, −55°). In Figure 2b–e, individual distributions of ϕ and χ_1 are shown for proline and hydroxyproline, respectively. The two residues prefer opposite pucker conformations, as is clear from the distributions of χ_1, and due to the ϕ–χ_1 correlation, this leads to different ϕ distributions. The insets in Figure 2d and e show the ϕ and χ_1 distributions obtained with the previous hydroxyproline parameters.[13] The previous parameters fail to capture the opposite pucker preferences, and thus yield very similar ϕ distributions for proline and hydroxyproline, which is what initiated the present work.

Molecular Dynamics Simulation of Collagen-Like Peptides

In this section we report on MD simulations of collagen-like peptides, $[(PPG)_{10}]_3$ and $[(POG)_{10}]_3$ (O denotes hydroxyproline), performed using the new hydroxyproline parameters. As the formulas indicate, these collagen-like peptides are homotrimers of 10 Pro-Pro-Gly and Pro-Hyp-Gly triplets, respectively. We analyze the pucker preferences of Pro/Hyp within the collagen triple helix in comparison with those of Pro/Hyp monomers, and discuss the effect of the hydroxylation.

Initial conformations of $[(PPG)_{10}]_3$ and $[(POG)_{10}]_3$ (capped with acetyl and N-methyl groups) were prepared with the gencollagen program[28] and were each simulated for 10 ns at a constant temperature of 300 K using the same MD simulation methods as in the preceding section except that Coulomb and van der Waals forces were truncated at a cutoff distance of 1.5 nm. First, 2 ns were considered to be an equilibration period and was not included in the analysis.

Figure 3 shows distributions of χ_1 and ϕ, recorded every picosecond from the trajectory of $[(PPG)_{10}]_3$, for prolines in the X and Y positions, respectively. To remove any end effect due to slight fraying, only four central triplets per chain were included in the histograms. For each chain, in other words, three triplets at the N-terminus and three at the C-terminus were excluded. For prolines in the X position, the distributions are very similar to those for the proline monomer. For prolines in the Y position, on the other hand, the pucker conformation is forced towards exo as can be seen from the χ_1 distribution, and thus the peak of the ϕ distribution is shifted significantly. This change in the pucker conformation is obviously induced by the triple helical structure and must be accompanied by an energetic cost.

Figure 4 shows the corresponding plots for $[(POG)_{10}]_3$. χ_1 and ϕ distributions for both prolines (in the X positions) and hydroxyprolines (in the Y position) overlap with those for the respective monomers. The energetic cost of shifting pucker conformations is now absent. Therefore, it is most reasonable to conclude that our MD simulations support the theory that the role of hydroxylation in collagen is to stabilize the triplex helix by adjusting to the right pucker conformation (and thus the right ϕ angle) in the Y position.

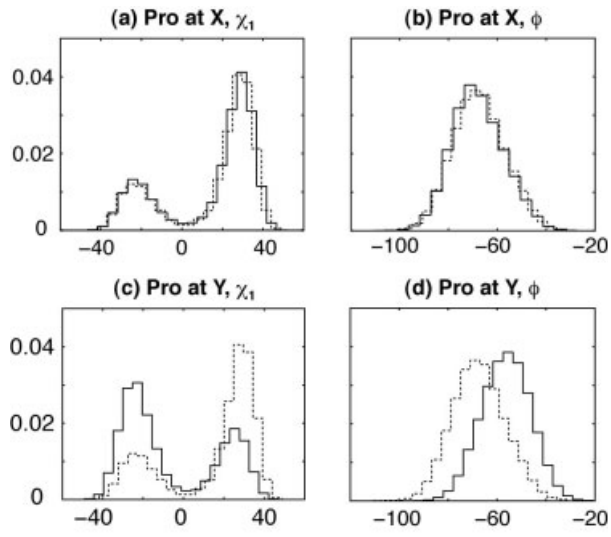

Figure 3. MD simulation of $[(PPG)_{10}]_3$. The normalized χ_1 and ϕ histograms are shown for prolines in the X and Y positions, respectively. For comparison, the χ1 and ϕ histograms for the proline monomer (Fig. 2) are shown as dashed lines.

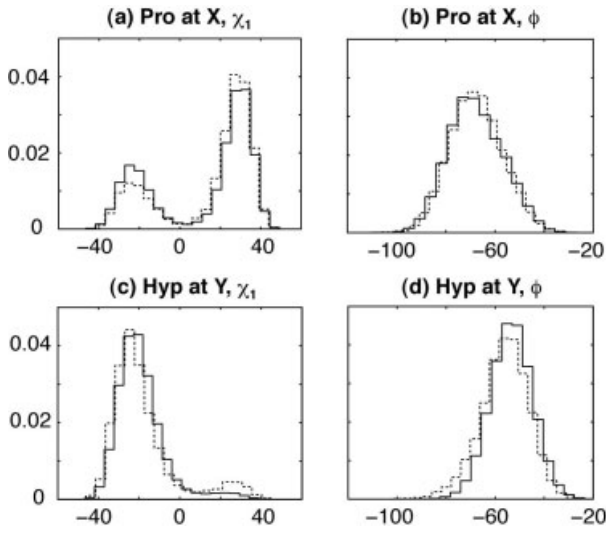

Figure 4. MD simulation of $[(POG)_{10}]_3$. The normalized χ_1 and ϕ histograms are shown for prolines (in the X position) and hydroxyprolines (in the Y position). For comparison, the $\chi 1$ and ϕ histograms for the Pro/Hyp monomers (Fig. 2) are shown as dashed lines.

Concluding remarks

We have developed a new set of molecular mechanics parameters for hydroxyproline that reproduces the correct pucker preference. Our MD simulations of collagen-like peptides using the new parameters support the importance of pucker conformations in the stability of the collagen triple helix. We believe the new parameters will be useful for computational studies of collagen.

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