Two Distinct Local Structures with Different Mechanical Characteristics in Osteogenesis Imperfecta-related Gly Substitutions Type I Collagen

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Statement of Purpose: Collagen exhibits specific structural and mechanical properties responsible for healthy joints and muscles in mammalian bodies. However, Gly substitution mutations in collagen's triple-helical domain undermine the ordered structure, leading to incurable and debilitating diseases, such as osteogenesis imperfecta (OI) in human type I collagen.[1] Specifically, changes in collagen's material behaviors and properties at the molecular scale are fundamental reasons inhibiting the functions of recognition and assembly.[2] Numerous studies using non-equilibrium molecular dynamics (MD) simulation have revealed the mechanical properties changes due to Gly substitution mutations.[3,4] However, the equilibrium structure-property relationships of wild-type and mutant collagen remain elusive. Moreover, systematically understanding the equilibrium mechanical behavior of OI-related mutant collagen renders deeper molecular insights into the mechanisms of genetic diseases, potentially providing a direct path for developing targeted, individualized molecular therapies. This project explores detailed local structural changes due to OI-related Gly substitution mutations in a selected human type I collagen sequence by combining equilibrium MD simulations and Markov state models (MSMs). Such structural changes further affect the axial, bending, and torsional stiffnesses, determined from natural fluctuations.

Methods: The sequence of the wild-type collagen is selected from human α-1(I) chain residues 1010-1051, together with another α-1(I) chain and corresponding α-2(I) chain, forming into a triple-helical structure. Triplets of GPP amino acids were added at both ends of the peptides to avoid potential side effects. Four OI-related mutated collagens were then generated by replacing Gly at the corresponding sites in α-1(I) chains. To study the natural dynamics of collagen, we performed an MD simulation with CHARMM 36m forcefield for each collagen structure in the NPT ensemble at 310 K for 100 ns after energy minimization, and the last 40 ns simulation with 2000 conformational ensembles were used for analysis. Based on the equipartition theorem,[5] naturally occurring thermal fluctuations of collagen molecules in equilibrium MD simulations are correlated with the axial, bending, and torsional stiffnesses. Besides, we constructed MSMs for each mutation to obtain equilibrated collagen structures to explore the structure-property relationship in collagen. To reduce the error of MSMs resulting from the scarcity of the data in 2,000 ensembles, we re-obtained 40,000 ensembles in 4×100 ns for each mutation.

Results: Although all collagens unwound at the mutation sites, the subtle structural changes were very different, leading to different structural and mechanical properties, as shown in Fig. 1 and Table 1. Two distinct local structures with different mechanical characteristics among different Gly substitution sites. With mutations in the 1022nd site, the stable short β-structures with new H-bonds formed, resulting in a more stable local state with a 20 % increase in axial and torsional stiffness than the wild-type collagen. However, mutations in the 1025th site disrupted existing H-bonds, leading to an erratic unwound structure with more intensive fluctuations, and thus, reduced all stiffnesses by over 20 %. The formation of most new H-bonds primarily involved Hyp, Arg, Ser, and Asp amino acids, which have polar side chains that serve as the donors and acceptors. Therefore, the side chains near the mutation sites may govern the formation of new H-bonds, affecting local structures and stiffnesses. Despite these differences, the mutated collagens exhibited more irregular motion than the wild-type collagen in simulations.

Table 1. Stiffnesses of the wild-type and OI-related mutated collagens.

Cases (pN/nm)	Axial stiffness	Bending stiffness	Torsional stiffness
Wild type	504.098 ± 12.726	0.528 ± 0.015	7.812 ± 0.084
1022nd Gly to Val	625.089 ± 33.042 (24.001%)	0.538 ± 0.018 (1.894%)	9.356 ± 0.333 (19.76%)
1025th Gly to Arg	399.710 ± 7.152 (-20.708%)	0.388 ± 0.009 (-26.515%)	5.774 ± 0.135 (-26.089%)
1049th Gly to Ser	473.541 ± 20.619 (-6.062%)	0.565 ± 0.012 (7.008%)	6.635 ± 0.078 (-15.067%)
1022nd and 1025th	535.427 ± 18.195 (6.215%)	0.446 ± 0.009 (-15.530%)	9.398 ± 0.297 (20.302%)

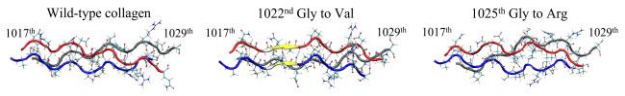

Fig. 1. The local structures with residues 1017-1029 of the wild-type and the 1022nd and 1025th site mutation collagens.

Conclusion:
This study harnesses the natural fluctuations in equilibrium MD simulations to calculate the stiffnesses of wild-type collagen and several OI-related Gly substitution mutations. Remarkable differences in the stiffnesses for various mutated collagen were attributed to the unwound structural characteristics after mutations. The analytical methods in this project will also benefit and impact scientists who investigate the structural traits and mechanical properties of scleroprotein in general.

References:

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