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The effect of point mutations on structure and mechanical properties of collagen-like fibril: A molecular dynamics study

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ABSTRACT

Understanding sequence dependent mechanical and structural properties of collagen fibrils is important for the development of artificial biomaterials for medical and nanotechnological applications. Moreover, point mutations are behind many collagen associated diseases, including Osteogenesis Imperfecta (OI). We conducted a combination of classical and steered atomistic molecular dynamics simulations to examine the effect of point mutations on structure and mechanical properties of short collagen fibrils which include mutations of glycine to alanine, aspartic acid, cysteine, and serine or mutations of hydroxyproline to arginine, asparagine, glutamine, and lysine. We found that all mutations disrupt structure and reduce strength of the collagen fibrils, which may affect the hierarchical packing of the fibrils. The glycine mutations were more detrimental to mechanical strength of the fibrils (WT > Ala > Ser > Cys > Asp) than that of hydroxyproline (WT > Arg > Gln > Asn > Lys). The clinical outcome for glycine mutations agrees well with the trend in reduction of fibril's tensile strength predicted by our simulations. Overall, our results suggest that the reduction in mechanical properties of collagen fibrils may be used to predict the clinical outcome of mutations.

1. Introduction

Collagen is the most abundant protein in mammals and is a key component of the extracellular matrix, supporting and connective tissues. Collagen, like many biological materials, has complex hierarchical structure where tropocollagen molecules assemble into a parallel, overlapping staggered array to form collagen fibrils [1]. Diseases associated with collagen often have detrimental consequences, since it is an integral part of the mechanical veracity of the majority of the structural constituents in organisms. For example, osteogenesis imperfecta (OI), or brittle bones disease, involves a large deficiency in collagen, resulting in stunted growth, easy bone fracture or breakage, thin skin, and weak tendons, among other symptoms [2]. The disease varies in severity from mild to lethal, depending on the type and location of the mutation, and affects about 1 in 20,000 people worldwide.

Type I collagen is found in the highest quantity in the human body and its deficiency is the cause of most collagen diseases. Type I collagen polypeptides have a Glycine(Gly)-X-Y repeat unit, where glycine is required to ensure the formation of the triple helix, and the X and Y positions are most commonly occupied by proline and hydroxyproline [1,3]. The Gly-Pro-Hyp tripeptide unit is the most stabilizing [4], as well as the most common repeat sequence in fibrillar type collagens. Amino acid mutations in collagen sequence modify the intermolecular properties of both the tropocollagen molecule and the fibril and lead to formation of a defective connective tissue [5]. It is known that a defect or mutation in the molecular structure of collagen modifies the behavior and properties of the molecule; however, there is a long standing debate on the mechanism for the cause of OI itself. Possible explanations include the deprivation of molecular stability and correct association with other collagen molecules, as well as the lacking of essential binding sites [6]. A further understanding of the effect of point mutations in type I collagen is necessary to gain perspective in this disease.

Lethal OI mutations most typically involve single point amino acid mutations [6], which can lead to a creation of a bulge in collagen, affecting molecular mechanics of the collagen fibril and interactions with other molecules [7]. The majority of OI mutations involve a mutation of glycine, which is buried at the center of the triple helix, to a larger amino acid [6,8,9]. Specifically, it has been determined that mutation of glycine to alanine is 19% lethal, serine is 30%, cysteine is 39% and aspartic acid is 68% [9]. The order of destabilization of tropocollagen was determined to be Ala<Ser<Cys<Arg<Val<Glu<Asp<Trp which correlates well with the clinical outcomes [10].

The effect of mutations of proline and hydroxyproline amino acids, which are exposed to the surface, are much less investigated and the clinical effect are less characterized [11]. However, a single substitution of Hyp to Gln has been associated with a Marfan-like syndrome [12]. Persikov et al used a host-guest peptide approach to establish a scale of inclination for all amino acids to be present in the X or Y positions, putting a single varied "guest triplet" in the center of a tropocollagen [4]. They found that mutations at the X and Y positions to imino acids

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are stable while mutations to Gly and aromatic residues are destabilizing [4]. Recent molecular dynamics (MD) simulations of Hyp and Pro mutations in collagen indicated that mutations change the formation of interchain hydrogen bonds, solvent interactions, and puckering of neighboring imino acids and, thus, the structural stability of the collagen [13]. There has been a considerable research progress in elucidation of effect of amino acid mutations on collagen structure; yet the relation between structure of collagen and human disease remains unclear.

In this study, MD simulations were used to determine the effect of point mutations on stability and mechanical properties of collagen-like fibril containing the Gly-Pro-Hyp repeat unit. Previous MD simulations studies of tropocollagen have been successful in investigating the structural aspects of the protein and explaining experimental results [6,7,14–18]. For example, multiscale simulations indicated that severity of OI phenotype correlates well with the mechanical properties of glycine residue mutations [7]. Simulations of mechanical testing of tropocollagen molecule with Gly mutations were successful in correlation of the loss of molecular stiffness with the implications of the disease [19]. While previous simulations of collagen mutations were performed on tropocollagen molecules, it is not known to what extent and at what level in a complex hierarchical structure of collagen the mutations influence the properties. Thus, in this study we focus on the effect of mutations on the fibril structure. The aim of our simulations is to compare the effects of Hyp and Gly mutations on fibril mechanical properties. Glycine is located on the interior axis of the tropocollagen helix, where there is no room for a larger side group, whereas proline and hydroxyproline point outward, stabilizing the triple helix. In our first set of mutations, a glycine residue was mutated to alanine, aspartic acid, cysteine, or serine amino acid. In the second set of mutations, a hydroxyproline was mutated to arginine, asparagine, glutamine, or lysine amino acid (Fig. 1).

2. Materials and methods

Generally, prediction of protein stability due to mutations can be accomplished by using methods from four categories: (1) physical or first principle methods, (2) empirical potential functions, (3) machine learning methods, and (4) statistical potential methods. Physical methods are the most accurate and the most computationally expensive. For our study, we chose physical methods that rely on knowledge of atomistic models and the use of molecular dynamics, molecular mechanics and/or Monte Carlo simulations. In these methods the mutations are usually introduced into a folded protein structure and simulations with carefully parameterized force field are used to assess the changes in protein stability, which provide quantitative insights into molecular scale changes associated with mutations [6,13,16,19,20]. The limitations of the physical methods include sampling difficulty of mutated structures, unknown folding pathway, length and size of simulations, and accuracy of the force fields.

In this study, a 15 amino acid homotrimer tropocollagen molecule was built (Fig. 1) based on the molecule from the Protein Databank with PDB ID 1QSU [21]. The length of the tropocollagen and fibril used in our study is comparable with the length used in experimental studies [4]. The intermolecular and intramolecular bonding patterns of this homotrimer are analogous to normal type I collagen, making analysis of this structure resonate with true collagen. Single point mutations were introduced into the wild-type molecule per specifications listed

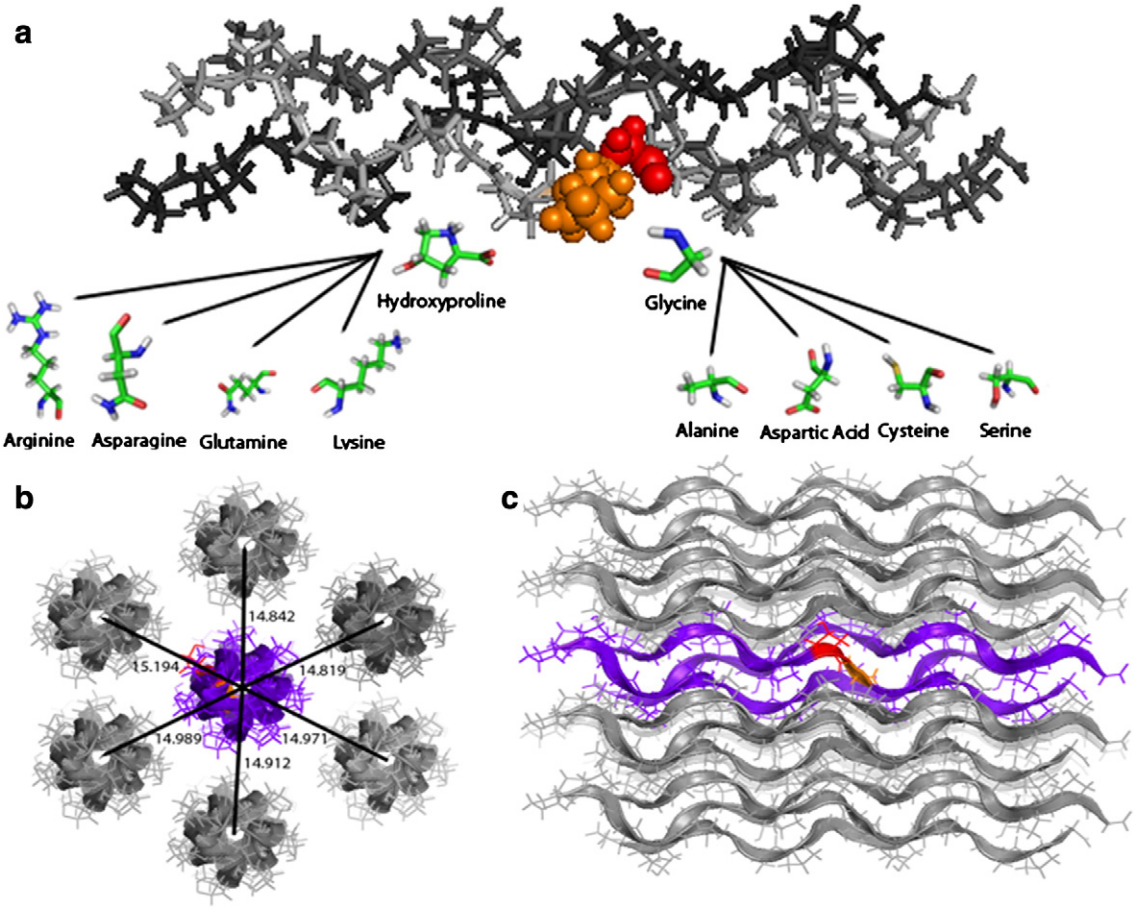

Fig. 1. (a) The tropocollagen molecule is shown with hydroxyproline highlighted in orange and glycine highlighted in red. Each mutation is shown for substitution with either hydroxyproline or glycine. (b and c) End and side view of the initial fibril structure where only the central tropocollagen had a point mutation, and the distances from the centroid of each of the outer tropocollagen molecules to the centroid of the central one (in purple) are shown in Angstroms.