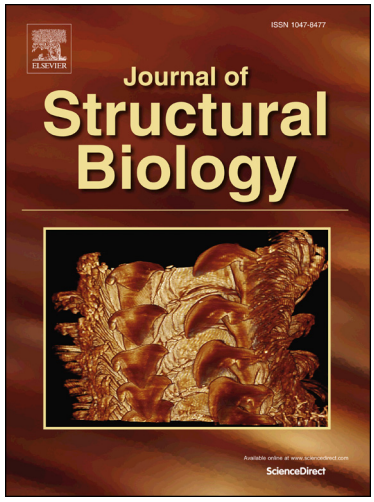

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Collagen Gly missense mutations: Effect of residue identity on collagen structure and integrin binding

Yimin Qiu[1], Arya Mekkat[2], Hongtao Yu[1, 2], Sezin Yigit[1], Samir Hamaia[3], Richard W. Farndale[3], David L. Kaplan[1], Yu-Shan Lin[2*], and Barbara Brodsky[1*]

[1]*Department of Biomedical Engineering, Tufts University*
[2]*Department of Chemistry, Tufts University*
[3]*Department of Biochemistry, University of Cambridge, Cambridge UK*

[*]*Corresponding authors*

Current Address:

Sezin Yigit, Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06520

Keywords: Osteogenesis Imperfecta; collagen; missense mutation; integrin binding sites; molecular dynamics, recombinant protein expression; triple-helix.

Highlights: The severity of the bone disorder Osteogenesis Imperfecta is influenced by the specific residue which replaces Gly in the repeating (Gly-Xaa-Yaa)$_n$ tripeptide sequence of type I collagen. A recombinant bacterial collagen system and molecular dynamics simulations were applied here to investigate the influence of Ala, Ser and Val replacing Gly within the integrin binding collagen region. The degree of local distortion of the triple-helix and the disruption of integrin binding were affected by the replacement residue identity at some but not all sites, and the implications for collagen diseases are discussed.

ABSTRACT: Gly missense mutations in type I collagen, which replace a conserved Gly in the repeating (Gly-Xaa-Yaa)$_n$ sequence with a larger residue, are known to cause Osteogenesis Imperfecta (OI). The clinical consequences of such mutations range from mild to lethal, with more serious clinical severity associated with larger Gly replacement residues. Here, we investigate the influence of the identity of the residue replacing Gly within and adjacent to the integrin binding ^{502}GFPGER^{507} sequence on triple-helix structure, stability and integrin binding using a recombinant bacterial collagen system. Recombinant collagens were constructed with Gly substituted by Ala, Ser or Val at four positions within the integrin binding region. All constructs formed a stable triple-helix structure with a small decrease in melting temperature. Trypsin was used to probe local disruption of the triple helix, and Gly to Val replacements made the triple helix trypsin sensitive at three of the four sites. Any mutation at Gly505, eliminated integrin binding, while decreased integrin binding affinity was observed in the replacement of Gly residues at Gly502 following the order Val > Ser > Ala. Molecular dynamics simulations indicated that all Gly replacements led to transient disruption of triple-helix interchain hydrogen bonds in the region of the Gly replacement. These computational and experimental results lend insight into the complex molecular basis of the varying clinical severity of OI.

1. Introduction

Collagen, the most abundant protein in vertebrates, is crucial to the structural integrity and mechanical stability of connective tissues, including skin, tendon, cartilage, cornea and bone. The collagen triple-helical domain, a common structural motif of all types of collagen, is composed of a repetitive (Gly–Xaa–Yaa)$_n$ amino acid sequence, where the Xaa and Yaa positions are often occupied by imino acid residues, proline and hydroxyproline (Brodsky and Persikov, 2005). As the smallest amino acid residue, Gly is required at every third position in the polypeptide because it is the only residue capable of fitting into the core of the triple helix (Ramachandran and Kartha, 1955; Rich and Crick, 1961). Up to 28 collagen types have been identified in human and can be categorized into two general groups, fibrillar (e.g. types I, II, III, V and XI) and non-fibrillar collagens. Among the 28 types of human collagen, type I is the most abundant and is the major protein in bone. Type I collagen is natively heterotrimeric and consists of two α1(I) chains and one α2(I) chain, encoded by the COL1A1 and COL1A2 genes, respectively.