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Modelling collagen diseases:

STRUCTURAL BIOLOGY

Barbara Brodsky and Jean Baum

Barbara Brodsky is in the Department of Biochemistry, University of Medicine and Dentistry of New Jersey — Robert Wood Johnson Medical School, Piscataway, New Jersey 08854, USA. Jean Baum is in the Department of Chemistry and Chemical Biology, BioMaPs Institute, Rutgers University, Piscataway, New Jersey 08854, USA. brodsky@umdnj.edu; jean.baum@rutgers.edu

Abstract

Mutations in collagen lead to hereditary disorders such as brittle-bone disease. Peptide models for aberrant collagens are beginning to clarify how these amino-acid replacements lead to clinical problems.

Collagen is the predominant protein in the body defining the mechanical properties of tissues. In many hereditary connective-tissue disorders, collagen's regular repeating sequence of amino acids is disrupted. Short peptide chains have proved to be valuable models in understanding both these pathologies and normal collagens¹. In the *Journal of the American Chemical Society*, Gauba and Hartgerink² report an intriguing peptide model for osteogenesis imperfecta, a dominant hereditary disorder commonly known as brittle-bone disease. The model allowed them to make disease-related mutations in any or all of the three peptide chains that make up collagen.

Collagen's molecular structure consists of three helical polypeptide chains coiled around each other to form a triple helix. The close packing of these chains creates a precise stagger in their alignment and requires that the smallest amino acid, glycine, occupies every third position in each peptide. The sequence must also have a high content of proline and its modified variant hydroxyproline. Some collagens comprise three identical chains, whereas others contain chains of differing amino-acid composition.

Type I collagen, which is composed of two identical chains and a third with a different amino-acid sequence, is the major protein in bone. Osteogenesis imperfecta is caused by mutations in any of the chains (Fig. 1), which change one of the glycines to a larger residue, such as serine. This delays folding of the protein and decreases the stability of the collagen molecules³. The folding defects can lead to retention of collagen within cells and their eventual death, and the structural alterations may result in defective binding of other components required for bone formation⁴. The size and complexity of entire collagen molecules make it difficult to interpret the structural basis of changes in stability and folding. Fortunately, small triple-helix peptide models offer the opportunity to vary amino-acid sequences, allowing the molecular details of changes in a mutation site and its interactions to be defined.

The Hartgerink group has developed a system⁵ involving the assembly of three different peptides to form a mixed trimer that represents the latest advance in collagen model design. Peptide models have long been an integral component of collagen structural studies⁶, beginning in the 1950s with simple polymers of glycine or proline, and progressing through peptides with strictly repeating amino-acid sequences to triple-helical peptides containing sequences from collagen itself. Peptides with glycine as every third residue and a high content of proline and hydroxyproline self-associate to form stable trimers with three identical chains, known as

homotrimers. But attempts to use chains with differing sequences did not produce stable mixed molecules, or heterotrimers. Strategies for forming heterotrimeric triple-helical peptides similar to type I collagen have thus far focused on forcing the desired chain composition and alignment through covalent linkages between the peptides^{7–9}. Gauba and Hartgerink² have now revisited the idea of creating peptides with different sequences that, when mixed, form stable collagen-like trimers. Instead of being held together by covalent bonds, the authors' design relies on electrostatic interactions between the chains to assemble and align the peptides into a triple helix.

In earlier studies, Gauba and Hartgerink⁵ developed an optimal design for assembling and stabilizing heterotrimeric collagen-like peptides by using equal amounts of three different peptides: a neutral peptide consisting of ten repeats of a tripeptide made of proline, hydroxyproline and glycine; a positively charged version in which all of the hydroxyprolines are replaced by the basic amino acid lysine; and a negatively charged variant in which the prolines are replaced by the acidic amino acid aspartic acid. The complementary electrostatic charges on the three peptides lead to the formation of stable trimers with one chain of each type aligned as in native collagen.

In the new study², the authors have extended their strategy by incorporating segments of type I collagen sequence into the middle of the model peptides. These inserted sequences carry the osteogenesis imperfecta glycine-to-serine mutation in none, one, two or all of the triple helix's chains (Fig. 1). The positive, negative and neutral sequences flanking the regions of collagen sequence are still able to drive assembly of the peptides into collagen-like heterotrimers.

The authors' measurements of trimer-to-monomer thermal transitions show that single mutation substantially reduces the stability of these peptides, whereas subsequent mutations lead to less drastic changes. Their experimental results are in good agreement with computational studies¹⁰, which calculated the effect of introducing such mutations into a collagen triple helix. When the authors studied the folding of the peptide trimers, they saw the same trend; the first glycine mutation creates the biggest delay in triple-helix folding. Using Gauba and Hartgerink's approach, it should also be possible to vary the sequence around the mutation site within these peptides, as well as the identity of the amino acid replacing glycine, to investigate factors affecting the severity of osteogenesis imperfecta.

Many other disorders also arise from glycine mutations in collagen, although the clinical symptoms depend on the function and location of the type of collagen harbouring the mutation. For example, Alport syndrome results in progressive kidney disease due to mutations in type IV collagen, whereas Ehlers–Danlos syndrome type IV affects blood vessels as a result of mutations in type III collagen. All such diseases could, in principle, be investigated using the authors' approach.

The techniques used by Gauba and Hartgerink, based on circular dichroism spectroscopy, do not provide high-resolution structural information. But other self-assembling homotrimer triple-helical peptides have proved amenable to molecular-level studies by nuclear magnetic resonance and X-ray crystallography^{1,11}. It will therefore be exciting to see whether these self-assembling heterotrimeric peptides² can be used to directly visualize the structural perturbation in a collagen disease and provide a basis for rational design of therapeutic drugs. These more realistic peptide models of collagen could also reveal how mutations affect the formation higher-order structures and interactions with the other components of bone.

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Figure 1. Collagen mutations mimicked by peptide models

a, Type I collagen is a trimer, composed of two protein chains with identical amino-acid sequences (blue) and one chain with a different sequence (green). Non-triple-helical sequences at the carboxy-terminal ends direct the chain assembly of the molecule. Mutations of the amino acid glycine to serine (red dot) within the helical region result in osteogenesis imperfecta (OI), a dominant genetic bone disease. **b**, Peptides designed by Gauba and Hartgerink² incorporate a region of type I collagen sequence between flanking regions with repeating sequences that are positively charged, negatively charged or neutral. The repeating sequences direct trimer formation through electrostatic interactions between the chains. A glycine-to-serine mutation (red) mimics OI. Peptide sequences are given in single-letter amino-acid code, with O used to represent hydroxyproline.