Recombinant Human Collagens and Associated Reagents

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ABSTRACT

Among the largest and most abundant macromolecules in mammals, Type I Collagen interacts with a variety of molecules. It is synthesized as a pro form that undergoes multiple post-translational modifications such as proteolytic processing of its N- and C-propeptides by Procollagen N-proteinase (PNP) and Procollagen C-Proteinase (PCP, also known as BMP-1), respectively. While mature Type I Collagen from several species can be purchased, no pro form has been commercially available until now. We present preliminary analysis of recombinant human procollagens encoded by the COL1A1, COL2A1, and COL3A1 genes. Expressed by mammalian cells, these three purified proteins contain both the pro form and the C-propeptide. In addition, COL1A1 and COL2A1 contain the pN fragment. Both the pro form and the C-propeptide form disulfide-linked oligomers and contain N-linked carbohydrates. The pro form can be used to assess the activity of PNP, PCP, and PCP Enhancer 1 (PCPE1), which increases PCP activity. These recombinant proteins can also be used to study their binding partners, such as Pigment Epithelium Derived Factor (PEDF). We show recombinant human COL1A1 and COL2A1 binding equally to PEDF. In addition, these recombinant proteins have been used as immunogens to generate monoclonal and polyclonal antibodies that exhibit distinct specificity. In summary, the availability of recombinant human procollagens and associated reagents should facilitate future studies that focus on the biosynthesis, structure, function, and regulation of collagens.

Table 1. List of Collagens and Collagen-related Molecules Referenced in this Study.

Gene	Protein	Description
COL1A1	Type 1 Collagen, α1 chain	• Type I Collagen is a fibril-forming collagen comprised of two α1(I) chains and one α2(I) chain. • It is found in most connective tissues including bone, cornea, dermis, and tendon. • Mutations in COL1A1 are associated with type I-IV Osteogenesis imperfecta.[1]
COL2A1	Type II Collagen, α1 chain	• Type II Collagen is a fibril-forming collagen comprised of three α1(II) chains. • It is the essential component of cartilage. • Mutations in COL2A1 are associated with type II collagenopathies.[1]
COL3A1	Type III Collagen, α1 chain	• Type III Collagen is a fibril-forming collagen comprised of three α1(III) chains. • It is found in most soft connective tissues including skin, lung, and the vascular system, often in association with Type I Collagen. • Mutations in COL3A1 are associated with type III and IV Ehlers-Danlos syndrome.[1]
BMP1	Bone Morphogenetic Protein-1 (BMP-1)/ Procollagen C-proteinase (PCP)	• BMP-1/PCP cleaves the C-terminal propeptide domains from Procollagens I-III. • Mutations in BMP1 are associated with autosomal recessive osteogenesis imperfecta.[2]
PCOLCE	PCP Enhancer 1 (PCPE1)	• PCPE1 binds to the C-terminal propeptide domain of Procollagens I-III and enhances cleavage of the procollagens by BMP-1/PCP.[3]
SERPINF1	Pigment Epithelium Derived Factor (PEDF)	• PEDF is a collagen-binding protein that has anti-angiogenic, anti-tumorigenic, and neurotrophic functions.[4,5] • The anti-angiogenic activity of PEDF is dependent on its binding to collagen.[6]
	Peptide N-glycosidase F (PNGase F)	• PNGase F cleaves asparagine-linked high mannose and hybrid and complex oligosaccharides from glycoproteins.

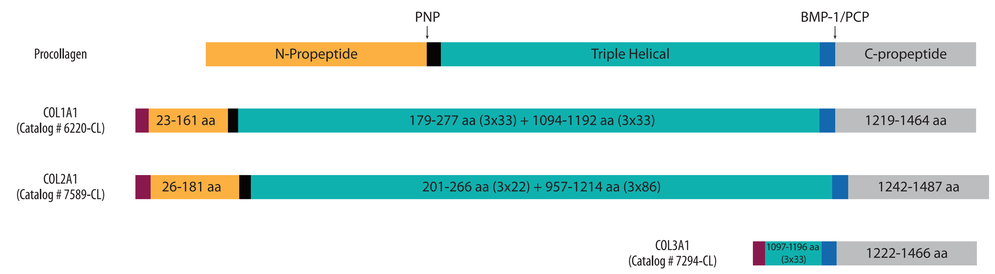

Figure 1. Domain Structure of the Native Procollagen Molecule and R&D Systems Recombinant Human Procollagen Proteins. Collagen proteins are synthesized as a procollagen molecule that contains a triple helical (TH) region (teal) composed of Gly-Xaa-Yaa triplets where Xaa and Yaa are frequently proline and hydroxyproline, respectively. The TH domain is flanked by N- and C-telopeptides (black and blue, respectively) and N- and C-propeptides (orange and gray, respectively). The propeptides are removed by PNP and BMP-1/PCP so that the mature triple helices can self-assemble into collagen fibrils. Removal of just the C-propeptide from the procollagen produces the pN fragment. R&D Systems Recombinant Human Pro-Collagen I α1 (COL1A1; Catalog # 6220-CL), Pro-Collagen II (COL2A1; Catalog # 7589-CL), and Pro-Collagen III α1 (COL3A1; Catalog # 7294-CL) proteins consist of shortened TH regions. The ranges of amino acid (aa) residues for the TH domain and the N- and C-propeptides are indicated for each recombinant human protein. The number of Gly-Xaa-Yaa triplets (number in parentheses) in the TH domain is also given. All recombinant human proteins contain an N-terminal 6X His tag (burgundy).

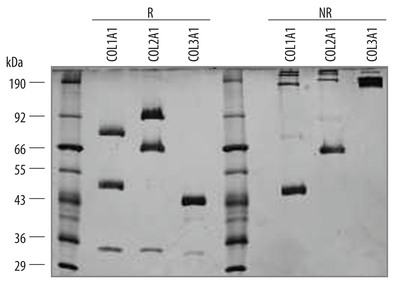

Figure 2. SDS-PAGE Analysis of Recombinant Human COL1A1, COL2A1, and COL3A1. Recombinant Human Pro-Collagen I α1 (COL1A1; Catalog # 6220-CL), Pro-Collagen II (COL2A1; Catalog # 7589-CL), and Pro-Collagen III α1 (COL3A1; Catalog # 7294-CL) were loaded on a 12% SDS-PAGE gel under reducing (R) and non-reducing (NR) conditions (1 µg/lane for all proteins). Proteins were visualized by silver staining. Under R conditions, all three recombinant procollagen proteins were separated into a monomer of the intact molecule (top bands) and the C-propeptide (bottom bands). The pN fragment (middle bands) of COL1A1 and COL2A1 was also separated out under R conditions. Under NR conditions, the monomers and C-propeptides remained bound, forming high molecular weight oligomers (>150 kDa, top bands). The pN fragment remained a monomer (bottom bands).

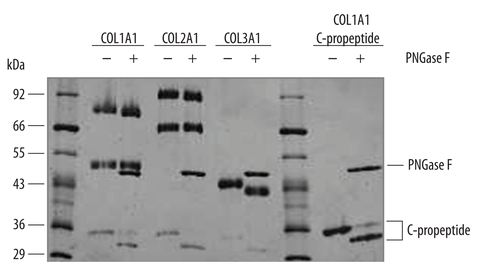

Figure 3. N-glycosylation of the C-propeptides of Recombinant Human COL1A1, COL2A1, and COL3A1 is Conserved. Recombinant Human Pro-Collagen I α1 (COL1A1; Catalog # 6220-CL), Pro-Collagen II (COL2A1; Catalog # 7589-CL), Pro-Collagen III α1 (COL3A1; Catalog # 7294-CL), and the C-propeptide from COL1A1 (COL1A1-C-propeptide) were treated with a recombinant PNGase F (+) or remained untreated (-). Samples were loaded on a 12% SDS-PAGE gel under reducing conditions (1 µg/lane for all proteins) and were visualized by silver staining. After PNGase F treatment, the molecular weights of the C-propeptides and intact molecules shifted; however, the pN fragment (middle bands) from COL1A1 and COL2A1 remained the same molecular weight. These results are consistent with the prediction that the only N-glycosylation site in all three recombinant human procollagen proteins is located in the C-propeptide region.

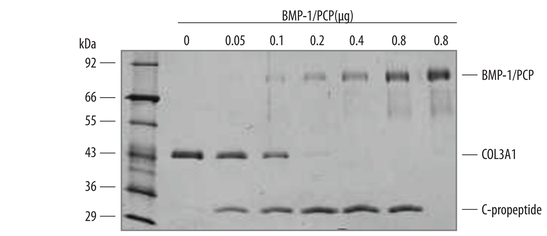

Figure 4. BMP-1/PCP Cleaves Recombinant Human COL3A1. Recombinant Human Pro-Collagen III α1 (COL3A1; Catalog # 7294-CL) was treated with increasing concentrations of Recombinant Human BMP-1/PCP (Catalog # 1927-ZN) for 1 hour at 37 °C. Samples were loaded (0.5 µg COL3A1/lane) on a 12% SDS-PAGE gel under reducing conditions and visualized by silver staining. The last lane contained only BMP-1/PCP. COL3A1 was completely cleaved by 0.2 µg BMP-1/PCP. BMP-1/PCP cleavage of COL3A1 produced an N-terminal fragment (not shown) and the C-propeptide. The C-propeptide was stable in the presence of excess amounts of BMP-1/PCP, indicating that BMP-1/PCP cleavage is highly specific.

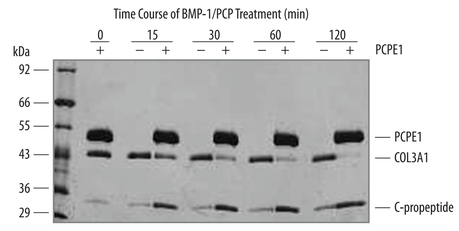

Figure 5. PCPE1 Enhances Cleavage of Recombinant Human COL3A1 by BMP-1/PCP. Recombinant Human Pro-Collagen III α1 (COL3A1; Catalog # 7294-CL) was treated with Recombinant Human PCPE 1 (100 µg/mL; Catalog # 2627-PE) for 30 minutes at 37 °C (+) or remained untreated (-). COL3A1 samples were then treated with Recombinant Human BMP-1/PCP (2.5 µg/mL; Catalog # 1927-ZN) at 37 °C for the indicated times. Samples were loaded (0.5 µg COL3A1/lane) on a 12% SDS-PAGE gel under reducing conditions and visualized by silver staining. In the presence of PCPE1, BMP-1/PCP cleaved more than 50% of COL3A1 in 15 minutes. In contrast, when PCPE1 was not present, BMP-1/PCP cleaved less than 50% of COL3A1 in 120 minutes.

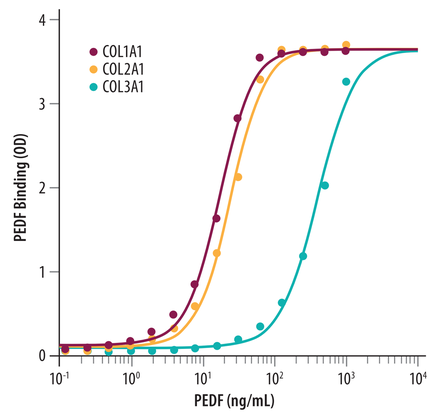

Figure 6. Recombinant Human COL1A1, COL2A1, and COL3A1 Bind to PEDF. Recombinant Human Pro-Collagen I α1 (COL1A1; Catalog # 6220-CL), Pro-Collagen II (COL2A1; Catalog # 7589-CL), and Pro-Collagen III α1 (COL3A1; Catalog # 7294-CL) were coated onto microplate wells at 5 µg/mL. Increasing concentrations of PEDF were added to the wells. Bound PEDF was detected using a Biotinylated Goat Anti-Human Serpin F1/PEDF Antigen Affinity-purified Polyclonal Antibody (Catalog # BAF1177) and visualized using Streptavidin-HRP (Catalog # DY998) and the Substrate Reagent Pack (Catalog # DY999). PEDF binds COL1A1 (burgundy line) and COL2A1 (orange line) with a similar affinity (BC_{50} = 20 ng/mL), and to COL3A1 (teal line), but at a lower affinity (BC_{50} = 400 ng/mL). PEDF binding of COL1A1 and COL2A1 is similar to its binding to bovine collagen I (not shown).

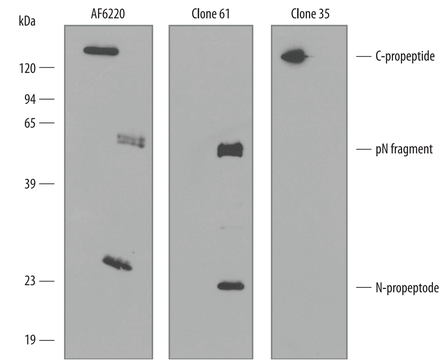

Figure 7. COL1A1 Antibodies Exhibit Distinct Specificities. Two mouse monoclonal antibodies (clones 35 and 61) and a Sheep Anti-Human Collagen I α1 Antigen Affinity-purified Polyclonal Antibody (Catalog # AF6220) were used to probe for Recombinant Human Pro-Collagen I α1 (COL1A1; Catalog # 6220-CL) by Western blot under non-reducing conditions. Clone 35 recognizes the C-propeptide of COL1A1 while clone 61 recognizes the N-propeptide and the pN fragment. The Human Collagen I α1 polyclonal antibody recognizes the C-propeptide, pN fragment, and N-propeptide.

CONCLUSIONS

- Recombinant human procollagen proteins, COL1A1, COL2A1, and COL3A1, were purified from the conditioned media of mammalian cells (Figure 1).
- All of these three proteins consist of a mixture of the pro form and the C-propeptide. COL1A1 and COL2A1 also contain the pN fragment (Figure 2).
- The pro form and the C-propeptide of each protein form disulfide-linked oligomers (Figure 2) and contain N-linked oligosaccharides (Figure 3).
- The pro forms can be used to assess the activity of procollagen processing enzymes (Figure 4) and regulators (Figure 5), such as PNP, BMP-1/PCP, and PCPE1.
- The recombinant human procollagen proteins can also be used to study their interactions with other molecules, such as PEDF (Figure 6).
- Specific monoclonal and polyclonal antibodies were raised against these functional proteins (Figure 7).
- The availability of recombinant human procollagens and associated reagents should facilitate future studies that focus on the biosynthesis, structure, functions, and regulation of collagens.

References

1. Kuivaniemi, H. *et al.* (1997) Hum. Mutat. **9**:300.
2. Martínez-Glez, V. *et al.* (2012) Hum. Mutat. **33**:343.
3. Steiglitz, B.M. *et al.* (2002) J. Biol. Chem. **277**:49820.
4. Ek, E.T. *et al.* (2006) Mol. Cancer Ther. **5**:1641.
5. Filleur, S. *et al.* (2009) J. Cell Biochem. **106**:769.
6. Hosomichi, J. *et al.* (2005) Biochem. Biophys. Res. Commun. **335**:756.

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