

# Collagen propeptides as indicators of collagen assembly

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## *Type I collagen is the main protein of the bone matrix*

It is old knowledge that bones are for about 70 % made up of calcium salts and for about 30 % of collagen. More specifically, almost all of the collagen in the collagen fibres of mineralized tissues is type I collagen, while most of the other almost 20 genetically distinct collagen types are essentially proteins of soft tissues. Since type I collagen is also the most abundant collagen type in soft tissues, it forms the vast majority of all the collagenous proteins in the body. It seems reasonable to assume that 50–70 % of the type I collagen in the body is in the skeleton. As bone is a metabolically more active tissue throughout life than most soft tissues, the contribution of bones to the biochemical markers reflecting type I collagen metabolism is even larger than is the proportion of this collagen type in the body.

Type I collagen is an early gene product in the development of the biochemical phenotype of the osteoblast (Stein et al. 1990). The maturation and mineralization processes of the matrix then require the activation of other genes, e.g. alkaline phosphatase and osteocalcin, respectively.

Type I collagen is a trimeric molecule, consisting of two identical and one different polypeptide chain, known as  $\alpha 1(I)$  chains and  $\alpha 2(I)$  chain, respectively (Table 1). The regulation of its synthesis must thus include the precise expression of two genes, located on two different chromosomes. A variant of type I collagen, the so called  $\alpha 1$ -homotrimer type I collagen, contains three identical  $\alpha 1(I)$  chains in the same molecule. A third variant, the so called onco-fetal, laminin binding collagen, has recently been described (Pucci-Minafra et al. 1993). Its structure is not known for certain, but it is possible that one  $\alpha 1$ -chain of type I collagen, one  $\alpha 1$ -chain of type III collagen and a third, acidic and so-far uncharacterized  $\alpha$ -chain form a triple-helical molecule. However, all the information available at present indicates that in most nonma-

lignant situations, irrespective of whether the synthesis rate of type I collagen is high or low, the classical form of type I collagen with two  $\alpha 1(I)$  chains and one  $\alpha 2(I)$  chain is synthesized almost exclusively.

## *Procollagen propeptides as indicators of collagen synthesis*

The original biosynthetic product is not type I collagen, but its precursor, known as type I procollagen and characterized by the presence of large extra domains at both ends of the rod-like collagen molecule (Figure 1). The functions of these domains, the procollagen propeptides, are related to chain assembly and prevention of premature fibre formation in the cells synthesizing the collagen. Once the molecule has reached the extracellular space, the propeptide parts are no longer needed and are subsequently cleaved off by the action of two specific endoproteases. The ensuing collagen molecules spontaneously assemble onto collagen fibres.

There is no indication of a further metabolism of the propeptides locally in the tissue, even though tissue macrophages may bear receptors capable of recognizing the propeptides. However, it is evident that during bone collagen synthesis the procollagen propeptides reach the circulation and can thus be meas-

Table 1. Suggested variants of type I collagen in humans

Variant	Chain composition	Occurrence
$\alpha 1$ -homotrimer	3 $\alpha 1(I)$	some tumors, transformed cells and inflamed tissues
classical	2 $\alpha 1(I)$ , 1 $\alpha 2(I)$	bone and soft tissues, the most abundant collagen in the body
onco-fetal, laminin binding	1 $\alpha 1(I)$ , 1 $\alpha 1(III)$ , 1 acidic $\alpha 1$ -chain <sup>a</sup>	some tumors and fetal tissues
<sup>a</sup> Suggested structure, not well characterized (Pucci-Minafra et al. 1993).		

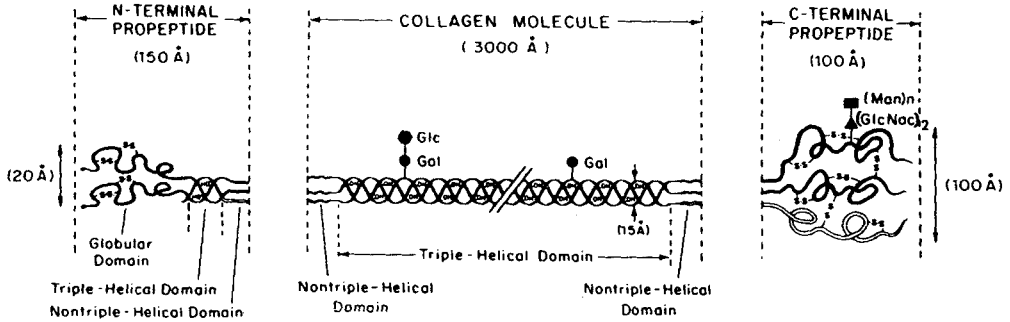


Figure 1. A classical type I procollagen molecule consisting of two  $\text{pro}\alpha 1(\text{I})$  chains and one  $\text{pro}\alpha 2(\text{I})$  chain. The aminoterminal propeptide (PINP) and the carboxyterminal propeptide (PICP) are marked. Reproduced with permission (Prockop et al. 1979).

ured in intact form in the blood. This review deals with the possibilities these proteins give for assessing the rate of bone collagen synthesis in a noninvasive manner in humans (Risteli and Risteli 1993, Eriksen et al. 1995).

Some general characteristics of both the aminoterminal and the carboxyterminal propeptide of type I procollagen are given in Table 2. When originating from classical type I procollagen, both propeptides are heterotrimers with two polypeptide chains derived from the original  $\text{pro}\alpha 1(\text{I})$  chains and one that was originally part of the  $\text{pro}\alpha 2(\text{I})$  chain. Despite the word propeptides, PINP and PICP are in fact due to their size proteins and, although derived from the

same gene product, quite different in chemical character. This also leads to the fact that the further metabolic fates of the two propeptides differ.

#### Aminoterminal propeptide of type I procollagen (PINP)

As the chain assembly takes place and subsequent winding into the native triple-helical conformation starts at the carboxyterminus of the procollagen molecule, the aminoterminal end of type I procollagen is the last part to assume its native conformation. Although there are no covalent bonds between the component chains in this region, the chains are held together by a short domain of collagenous triple helix in the middle of the propeptide part. The aminoterminal ends of the chains are obviously not in close contact with each other; in fact, the  $\text{pro}\alpha 2(\text{I})$  chain lacks the most aminoterminal domain, so-called Col 1 part. The Col 1 parts of the  $\text{pro}\alpha 1(\text{I})$  chains are the most immunogenic parts of the whole PINP propeptide.

PINP is cleaved off en bloc by a specific endoprotease in the extracellular space. However, in immunohistochemical studies PINP can often be visualized on thin collagen fibres in such areas of soft tissues where collagen synthesis is actively going on. Theoretically, a collagen molecule that has still retained its PINP part—known as type I pN-collagen—can fit onto a fibre, the PINP part filling the gap between two successive molecules. It is probable that this PINP is subsequently cleaved off to allow the fibre grow in thickness. In some situations the collagenous matrix could naturally also be broken down with the PINP still attached leading to the formation of antigenic Col 1 domains of the propeptide.

In serum the antigenicity of PINP is found in two peaks (Figure 2). The larger antigenic form corresponds to the authentic in vivo cleaved propeptide and the smaller resembles the aminoterminal Col1 domain of the  $\text{pro}\alpha 1(\text{I})$  chain. Several assays have

Table 2. Comparison of the aminoterminal and carboxyterminal propeptides of the classical human type I procollagen

	Intact PINP	PICP
Location	Aminoterminal	Carboxyterminal
Molecular weight	35,000	100,000
Shape	Elongated	Globular
Chemical nature	Phosphorylated, partially collagenous	Glycoprotein, high-mannose type
Related serum antigen		
Homogeneity	One major and one minor form	One form
Size	Same as PINP (major) or smaller, Col1-like (minor)	Same as PICP
Concentration ( $\mu\text{g/L}$ )	Men 20–76 Women 19–84	38–202 50–170
Clearance from blood	Scavenger receptor of liver endothelial cells	Mannose receptor of liver endothelial cells
Blocked by	Formaldehyde-treated albumin, Acetylated LDL, PIINP	Ovalbumin, mannan
Modulated by	No hormone sensitivity?	Hormone sensitive

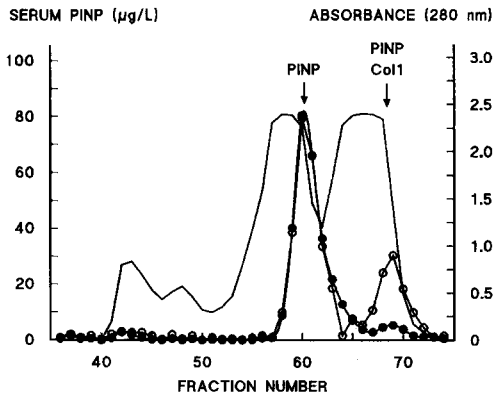


Figure 2. Gel filtration analysis of the PINP antigens in normal adult human serum. Thin continuous line = total protein content of the fractions; closed symbols = PINP measured by an equilibrium version of a PINP assay (Jukkola et al. 1993); open symbols = PINP measured by a sequential saturation version of the same PINP assay. The elution position of an intact PINP standard and that of the Col1 domain of a pro $\alpha$ 1(I) chain of PINP are indicated by the arrows.

Table 3. Assays for the aminoterminal propeptide of human type I procollagen

Assay type <sup>1</sup>	Source and nature of antigen	Specificity <sup>2</sup>	Reference
ELISA	Amniotic fluid, monomeric chain	D > I?	Teisner et al. 1992
ELISA	Linear synthetic peptide	(D, I)?	Ebeling et al. 1992
RIA	Linear synthetic peptide	D > I?	Linkhart et al. 1993
RIA	Cell culture medium, trimeric propeptide	I = D	Jukkola et al. 1993
ELISA	Amniotic fluid, monomeric chain	D > I?	Price et al. 1994
RIA	Pleural fluid, monomeric Col 1 domain of pro $\alpha$ 1(I) chain of PINP	D > I	Melkko et al. unpublished
RIA <sup>3</sup>	Pleural fluid, trimeric $\alpha$ 1-homotrimer and classical propeptide	I >> D	Melkko et al. unpublished

<sup>1</sup> ELISA = enzyme-linked immunosorbent assay, RIA = radioimmunoassay

<sup>2</sup> Specificity based on the nature of the antigen, not necessarily reported by the authors  
I = Intact propeptide,  
D = degradation product of the propeptide

<sup>3</sup> Commercially available through Orion Diagnostica, FIN-90460 Oulunsalo, Finland

been described for PINP which differ with respect to their ability to detect the two different endogenous PINP antigens in the blood (Table 3). Consequently, both the reference intervals obtained and the magnitude of the changes observed in pathological situations can vary, depending on the assay variant used.

Also the standardization of the results is arbitrary, since two different molecular weight forms affect the response. The biochemical basis of these methodological problems is the fact that part of PINP remains on newly-formed collagen fibres, and that the strongest immunogenic part of the molecule is of a sequential nature and resides in the most aminoterminal, so-called Col1 domain of the propeptide. During the degradation of tissue type I pN-collagen and possibly after further degradation of the propeptide itself these Col 1 domain related peptides are liberated into circulation. A similar, although even more complicated situation has been encountered in the assay for the aminoterminal propeptide of type III procollagen (PIIINP), which is homologous to PINP (for further discussion see Risteli and Risteli, 1995).

### Carboxyterminal propeptide of type I procollagen (PICP)

The carboxyterminal propeptide of type I procollagen is a glycoprotein with a molecular weight of about 100,000 and oligosaccharide side chains of the high mannose type on both its pro $\alpha$ 1(I) and pro $\alpha$ 2(I) component chains. Disulfide bonds join the subunits together to form a globular protein; these bonds were once essential in directing the correct assembly of the three pro $\alpha$  chains and initiating the formation of the triple helix of the collagen molecule proper.

The release of the bulky PICP part is considered a necessary prerequisite for normal deposition of the type I collagen molecules into collagen fibrils. Accordingly, circulating PICP can only originate from the synthesis of type I collagen, not from its degradation. For each molecule of collagen deposited in the tissues, one molecule of PICP is released.

The first assay for the carboxyterminal propeptide of human type I procollagen was described about 20 years ago (Taubman et al. 1974). In subsequent years, this method was used in several studies, but not made generally available. The interest in this method increased with the interest in osteoporosis and several assays are now commercially available. As only native PICP proteins are used as the reference antigens and as the endogenous antigen in the serum is homogenous, all the assays should in principle give similar results. If in doubt, it is useful to test the performance of the assay by chromatographing human serum with an elevated concentration of PICP on a gel filtration column and measuring the PICP concentration directly in the fractions. If the assay detects only PICP, there should be one distinct peak of antigenicity, corresponding to a molecular mass of about 100,000, in comparison with globular standards.

### Clearance of the propeptides from the circulation

In order to correctly interpret the concentration observed for any analyte in the blood, it is essential to know not only its formation and release from the tissue of origin, but also its further metabolism and clearance from the circulation. Due to their size (PICP), shape and negative charge (PINP), the procollagen propeptides are not lost into the primary urine in the glomeruli. As these proteins obviously have no function after being cleaved off from the procollagen, efficient systems have evolved to ascertain that the amino acids of the propeptides can be recovered and reutilized. Specific, separate clearance systems for effective recycling exist both for PINP and PICP.

PINP and its homologue PIIINP are taken up and metabolized by the endothelial cells of the liver, as shown by studies on the fate of the human proteins in rats and pigs. The extrahepatic degradation of PIIINP is of little importance (Jensen et al. 1993). It was recently shown that this clearance is a physiological function of the scavenger receptor of liver endothelial cells (Melkko et al. 1994). The signal for uptake is most probably a negative charge, which in PINP is due to covalently bound phosphate and in PIIINP due to tyrosine-O-sulfate (Jukkola et al. 1993).

In rats, human PICP is also taken up in the endothelial cells of the liver by a typical receptor-mediated process. Experiments with competing ligands have unequivocally identified this receptor to be the mannose receptor (Smedsrød et al. 1990).

Receptors similar to both the scavenger and the mannose receptor of the liver endothelial cells are expressed in macrophages, but for the time being there is no indication that the latter would be involved in the clearance of the procollagen propeptides. Interestingly, the mannose receptor of macrophages can be induced by several hormones.

In rare cases there can be hereditary defects in the clearance of PICP. We have encountered this phenomenon in about one out of several thousand Finnish subjects. In these cases, the circulating PICP concentration is bizarrely elevated, i.e. about ten-fold the upper limit of the age-adjusted reference range, and follows an autosomal dominant inheritance pattern (Sorva et al. 1994). Factors known to affect the circulating PICP concentration such as pregnancy or glucocorticoid treatment, still have their effect, although the order of magnitude of the concentration varies from normal. Our initial studies indicate that the glycosylation of the PICP molecules of these individuals is normal. We are currently testing the hypothesis that the mannose receptor has a genetic

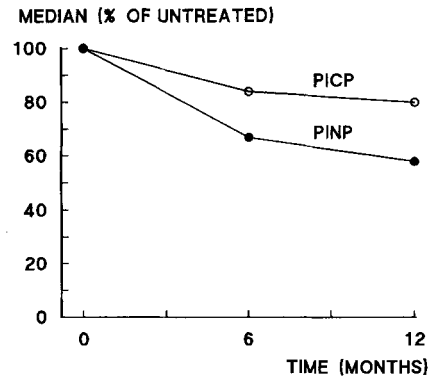


Figure 3. Median changes in the circulating concentrations of PICP and PINP in 49 post-menopausal women receiving hormone replacement therapy with estrogen and progestin (Suvanto-Luukkonen et al., unpublished data).

change in one or several of its carbohydrate binding domains.

### Comparison of PICP and PINP as indicators of type I collagen synthesis

Since the immunoassay for PICP has been commercially available for several years, there are numerous studies where it has been used (for reviews see Risteli and Risteli 1993, Eriksen et al 1995). In this review we concentrate on those, mostly unpublished studies, where both PICP and PINP have been analyzed simultaneously.

The intensive turnover of type I collagen taking place in growing bones and other tissues is reflected in the very high circulating concentrations of PINP and PICP in infants and children (Trivedi et al. 1991). The ratio PICP/PINP is around 0.5 in children and increases after puberty to adult value 2–3. During pubertal growth spurt the concentration of PINP increases more than that of PICP (Trivedi et al, unpublished data). Similarly after menopause the PINP concentration increases more than that of PICP and consequently the hormone replacement therapy decreases more the PINP than PICP concentration, e.g. after 12 months' treatment, 42% versus 20%, respectively (Figure 3). There are also 2–6-fold increases in PINP concentration in Paget's disease of bone (Sharp et al. unpublished data), in which situation the PICP assay often does not indicate similar changes. In breast carcinoma patients with bone metastases the serum PINP concentration may also increase more than that of serum PICP, the reasons for these differences are presently not known.

The intact PINP assay gives similar results as PINP Col 1 assay, which measures both the intact propeptide and its degradation product (Melkko et al.

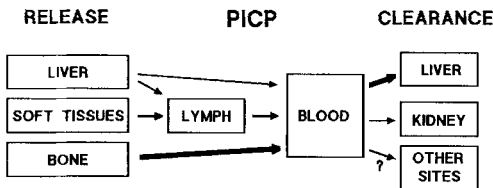


Figure 4. Origin and further metabolism of PICP. The thick arrows indicate the major routes.

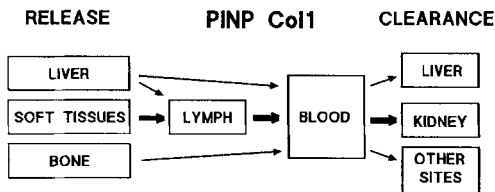
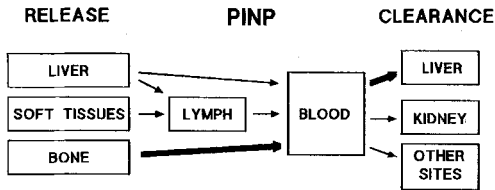


Figure 5. Origin and further metabolism of intact PINP and its degradation product resembling the Col 1 domain. The thick arrows indicate the major routes.

unpublished). However, since the degradation products have different origin and clearance route, it is possible that some individuals may have increased PINP Col 1 concentration due to increased soft tissue turnover or unnoticed kidney impairment. This was evident in one healthy subject, whose PINP Col1 concentration was clearly higher than expected on the basis of intact PINP concentration. This discrepancy is much larger in pathological conditions such as in patients on hemodialysis due to renal failure or in patients with catabolic status.

## Conclusions

The concentration of any marker of connective tissue metabolism in serum depends on the extent and timing of the release from the tissues and the route and mode of its clearance from the blood. Serum PICP antigen is mainly released from the synthesis of type I collagen in the bones and cleared by the liver (Figure 4). The extrahepatic clearance of PICP has not yet been excluded. The intact serum PINP antigen is mainly derived from the bones and cleared by the liver, whereas the serum PINP Col1 antigen is most

likely to great extent derived from the degradation of tissue type I pN-collagen and cleared by the kidneys (Figure 5). The extrahepatic degradation of intact PINP is not likely.

The propeptides of type I procollagen are among the analytes in clinical chemistry for which the metabolic fates are known in detail. In contrast to these, the further metabolism and clearance from the blood of other bone markers e.g. alkaline phosphatase still remain unknown. Thus the use of procollagen assays has a clear advantage during clinical evaluation of the results.

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