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Milan Adam

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Introduction

The editors feel that a brief explanation to the potential reader is in order. This volume is not intended to be an exhaustive treatment on connective tissue chemistry and biology. Neither is it simply a compilation of manuscripts resulting from a large international conference. We have tried to prepare a volume devoted to those branches of connective tissue research which are in the forefront of the scientific community at the present time. The papers were presented as plenary talks or introductions to workshops during the VIIth ECTC (Federation of European Connective Tissue Clubs) meeting in Prague (September 8–11th, 1980). The lectures were invited, and their selection was the result of discussions with the representatives of individual Connective Tissue Clubs throughout Europe. The editors and organizers of this meeting gratefully acknowledge this help. Special thanks go to Professor J. Scott of Manchester, England, and Professor L. Robert of Crèteil, France, for their interest, stimulating discussions, and highly qualified help.

Zdenek Deyl
Milan Adam

Prague, November 1980

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RECENT ADVANCES MADE IN INVESTIGATING THE MOLECULAR STRUCTURE OF COLLAGENS

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Over the past 20 years,investigations on the structure of the interstitial collagens,types I,II and III, has led to a detailed knowledge of the primary structure of collagen molecules (for review see Glanville and Kühn 1979)and has contibuted to a better understanding of collagen biosynthesis, helix formation,fibre formation,crosslinking and degradation processes.Amino acid sequences that have been completed are the α1(I) chain from calf and chick collagens and the α 2 and α 1(III)chains from calf collagen.Boththe human α 1(III)chain and calf α1(II) chain are near completion. The sequences of the amino terminal extension peptides of calf collagen proα1(I) and proα1(III) chains have been determined, also the carboxy terminal extensions of chick proα1(I) and proα2 chains, partly using classical amino acid sequencing techniques and more recently completed using neucleotide sequencing. The amino terminal extension of proα2 and pre-procollagen extensions are still under investigation.

Using the amino acid sequence of the triple helical region of type I collagen,calculations have been made to determine how molecules laterally and axially aggregate to form fibres (Hofmann,Fietzek and Kühn, 1978). The results are in good agreement with those derived from Xray diffraction studies. However, two models are still being discussed;one in which five molecules form a microfibril and a second in which the molecules are packed into a quasi hexagonal

structure. The formation of these higher structures is a self assembly process which appears to have a three step mechanism. The structure of the first aggregate formed is still not clear and the role the teleopeptides play in this process is unknown.The structure of the lysine derived reducible crosslinks in interstitial collagens have been elucidated (for review see Light and Bailey 1979) but the nonreducible crosslinks are still the subject of controversy.

Despite this detailed structural information, there remain some important questions concerning interstitial collagens that have yet to be answered. It is not known why an organism requires three interstitial collagens. clearly typeII is cartilage specific and the proportions of type I to type III in various tissues is important, but why ? The primary and higher strucures of these 4 collagen types are so similar, it would be expected that one molecule could substitute for another but this is not the case. Secondly, the in vivo assembly of the collagen triple helix is unclear. The mechanisms of chain selection, association and helix formation still require detailed investigation.

More recently, attention has turned to the basement membrane collagens. These do not appear to form fibrous structures, are very insoluble and , it was quickly realised, are pepsin sensitve.These characteristics are atypical for collagens and it is therefore expected that the collagenous structures in basement membranes are formed following different structural principles to those used for interstitial collagens. In general, the function of the interstitial collagens is to bestow mechanical strength on the extracellular matrix, this being done by forming long fibres of various diameters, either in the form of a meshwork (skin, bone) or parallel bundles (tendon). However, basement membranes have different functions depending upon the location of the membrane, for example, as a filtration barrier (placenta, kidney), scaffold in tissue repair and morphogenesis, a receptor site for cell membranes (epithelial cells) and as a support for the cytoskeleton of some cells (fat cells). None of these functions requires that the membrane has great mechanical strength.Whether the

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collagenous components play an active role in the functioning of a basement membrane or only a passive supportive role is unknown.

The major structural components that have been isolated from basement membranes are type IV collagen, type V collagen, 7s collagen, laminin and fibronectin (for review see Timpl and Martin 1981). The latter two glycoproteins are non collagenous and will not be further described here.

7s collagen has a similar amino acid and carbohydrate composition to that of type IV collagen (low Ala, high Hyl, Leu, Ile, Phe. 22% carbohydrate mostly Glc-Gal-Hyl) but differs in that it contains 15 to 20 Cys residues and 10 to 15 Tyr residues per 1000 amino acids (Risteli et al 1980). The molecule, which contains two structurally distinct domains, has a molecular weight of 360 000 daltons. The bacterial collagenase resistant domain has a molecular weight of 225 000 daltons, a mid point melting temperature Tm of 70° and contains 42 to 45 Cys residues per 1ooo amino acids. The bacterial collagenase sensitive domain has a Tm of 42° and very little or no Cys. The molecule has a complex subunit structure as, when reduced, several fragments are produced with molecular weights in the range 30 000 to greater than 300 000 daltons as shown in figure 1.

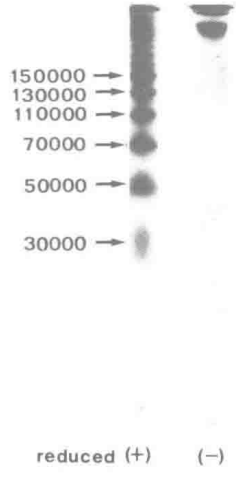

Figure 1. Disc electrophorisis gels (5%) stained with coomassie blue, showing 7s collagen (long form) isolated from a pepsin digest of human placenta.

The origin of this molecule is still uncertain. It may represent a new type of collagenous structure or is perhaps the crosslinking region of a type IV collagen complex which has been fragmented by the action of pepsin during the solubilisation of basement membrane components.

Type V collagen is found in both basement membranes (Furthmayr et al, 1980) and in the extracellular matrix (Burgeson et al.,1976. Brown et al., 1978). It resembles more the interstitial collagens as it contains one third Gly, is a pepsin stable molecule with a length of about 3oo nm and has a Tm of 37°. It has the molecular formular $[\alpha 1(V)]_2\alpha 2(V)$ (earlier $[\alpha B]_2\alpha A$) but unlike interstitial collagens does not readily form fibrils. Many of the mammalian collagenases that cleave interstitial collagens do not cleave type V. As type V collagen has only been isolated from pepsin solubilised tissue, the molecule as it is found in vivo has not been characterised. It also remains to be seen whether there is a procollagen molecule with structural similarities to type I procollagen and whether crosslinking regions are present. A third chain that has been observed in some preparations of type V, sometimes refered to as αC or $\alpha 3(V)$, has not been isolated as a native molecule and its relationship to type V is unclear (Sage and Bornstein 1979). The $\alpha 3$ chain appears to be tissue specific as demonstrated in figure 2.

Newly synthesised type IV collagen has been characterised in a number of cell and tissue culture systems (Tryggvason et al., 1980. Alitalo et al., 1980). After reduction and denaturation, two chains $\alpha 1(IV)$ and $\alpha 2(IV)$, have been identified with molecular weights of approximately 18o ooo and 165 000 daltons respectively. Although there does not seem to be any conversion of these products to smaller chains, it has not been possible to extract such products from basement membranes. The largest extractable chain described is 140 000 daltons and was isolated from a mouse tumor (Timpl et al.,1979). Because of the insolubility of basement membranes, it is necessary to solubilised their components using a proteolytic enzyme, usually pepsin. A typical preparation scheme

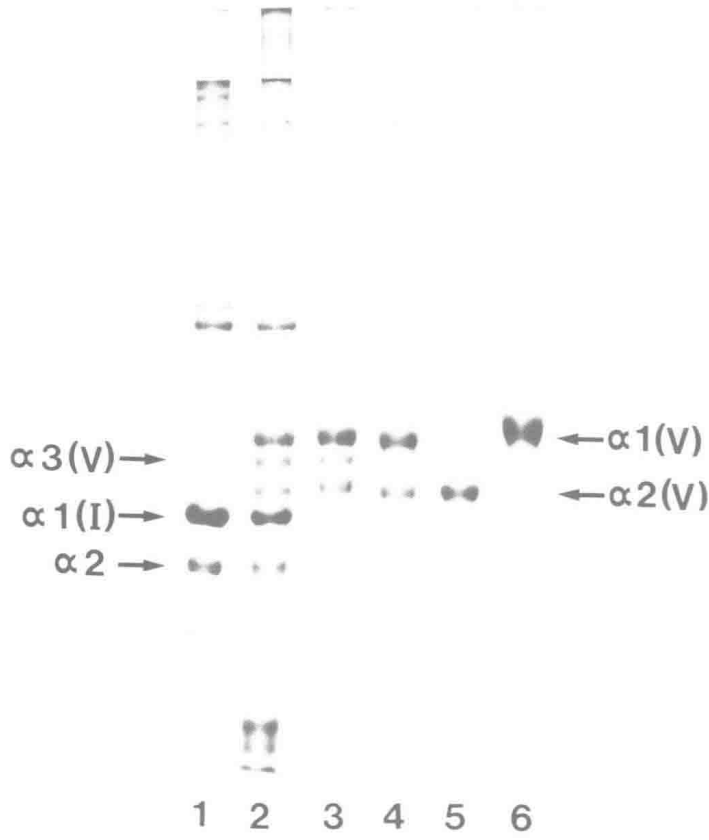

Figure 2. Stab electrophoresis gel (71/2%) stained with coomassie blue showing in lane 1, type I standard. Lane 2, pepsin solubilsed material from placental villi. Lane 3, type V isolated from material in lane 2. Lane 4, type V isolated from choreonic/ amneotic membrane of human placenta. Lane 5, α 2(V). Lane 6, α1(V).

for the collagenous components of human placental basement membrane is shown in figure 3. The type V, 7s and IV collagen fractions can be further purified by methods described in the literature (Bentz et al., 1978, Risteli et al., 1980, Glanville et al.,1979). The conditions for the first pepsin solubilisation are important as this determins the proportions of the various collagenous fractions and in the case of type IV collagen, the size of the fragments. To solubilise type V and 7s collagens, a temperature of

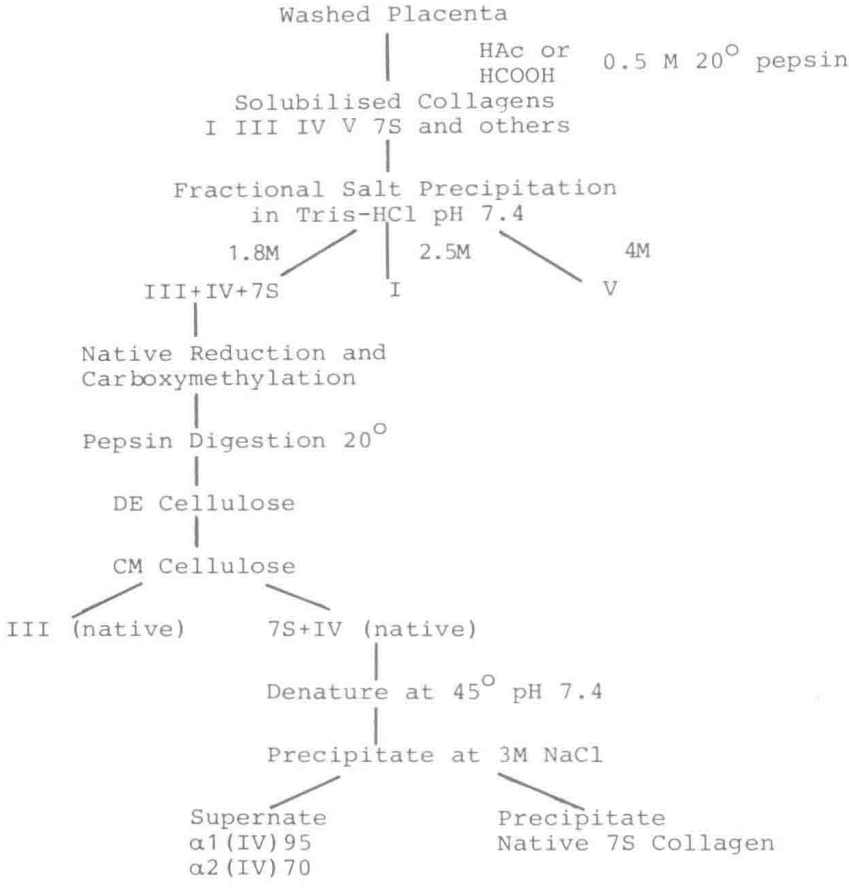

Figure 3. Scheme showing the separation of type V , 7s collagen and type IV collagen from pepsin solubilised human placental tissue.

20° and higher pepsin concentrations are necessary, whereas 4° pepsin solubilisation yields larger type IV collagen fragments but little type V and 7s. The size of the type IV fragments produced vary also with the animal species and tissue used. Such variations are illustrated in figure 4 for human placenta and bovine lens capsule type IV collagen prepared at 4° and 20°.

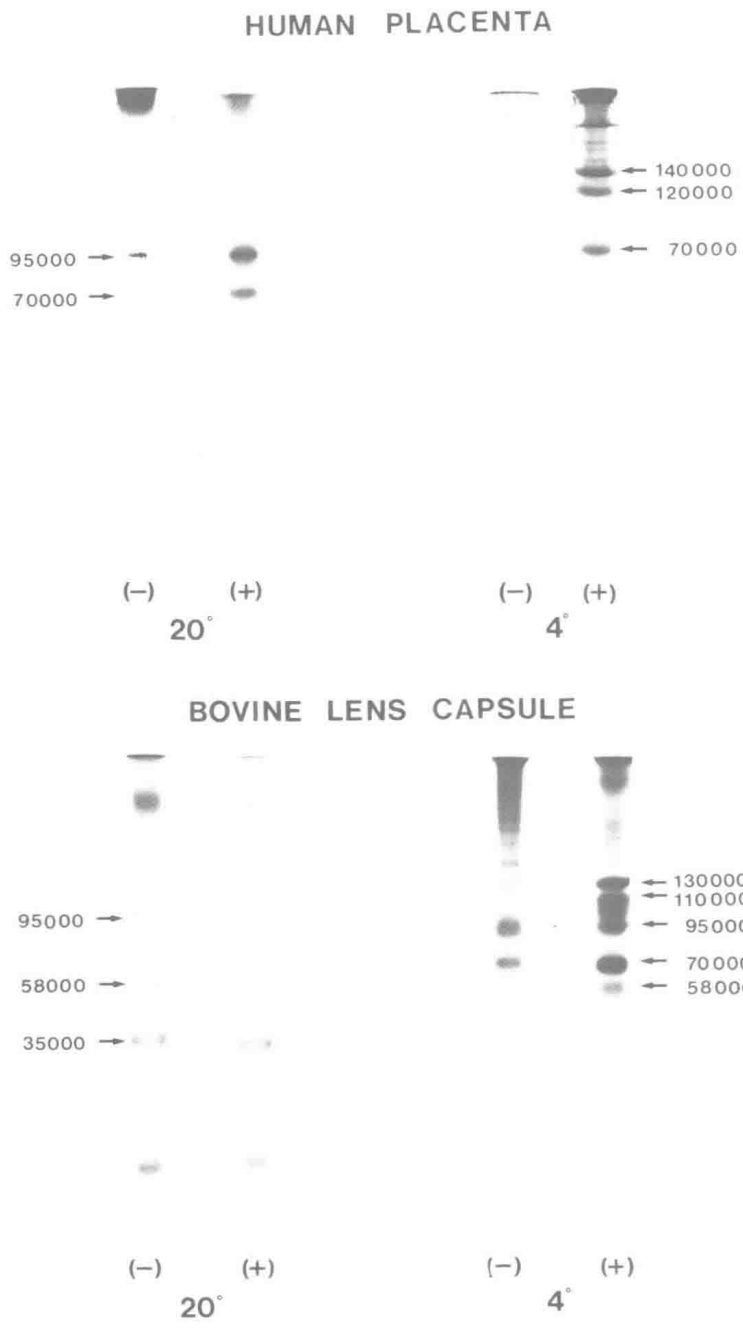

Figure 4. Disc electrophoresis gels (5 %) stained with coomassie blue showing pepsin fragments of type IV collagen isolated from human placenta and bovine lens capsule. The 20° pepsin solubilisation was carried out as shown in figure 3. In the 4° solubilisation, the native reduction, carboxymethylation and second pepsin digestion were omitted.(+) reduced (-) unreduced

Table 1 lists the pepsin fragments of type IV collagen that have been described in the literature and indicates the chain of origin of these fragments. It can be seen that fragments of approximately 140 000, 95 000 and 50 000 daltons form the α1(IV) chain have been isolated from many tissues and similarly 12o 000, 95 000, 75 000 and 5o ooo fragments from α2(IV) chain. This indicates that the type IV collagen in these tissues are structurally related. Mouse tumor and chick fragments are an exception to this generalisation as the major products appear to be around 50 000 daltons with no 95 000 or 70 000 dalton fragments.

Using the structural information available from investigations on human placenta, mouse tumor and bovine lens capsule type IV collagens, the pepsin fragments can be approximately aligned as shown in figure 5 .

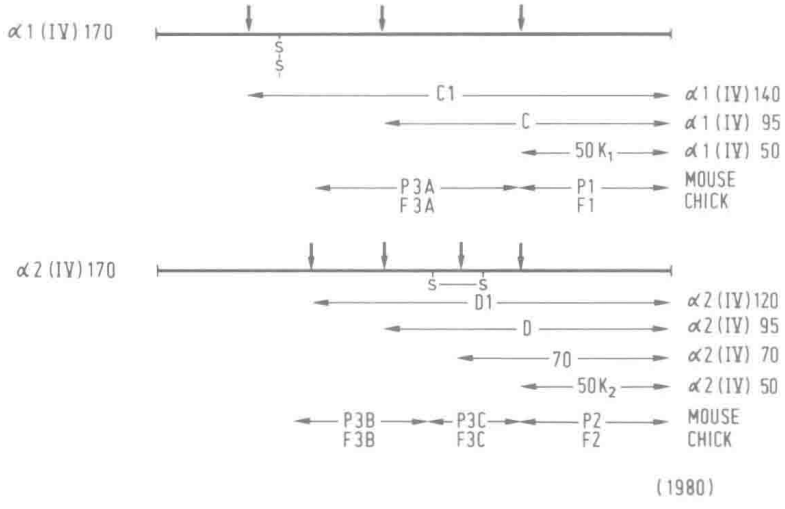

Figure 5. Scheme showing the alignment of human and bovine type IV collagen pepsin fragments in relation to the intact α(IV) chains. Nomenclature used in the literature is shown between the arrows and the fragment size to the right. Mouse and chick fragments are included for comparison. ↓ indicates the major pepsin cleavage sites.

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