CROSSLINKING OF COLLAGEN IN A HERITABLE DISORDER OF CONNECTIVE TISSUE: EHLERS-DANLOS SYNDROME

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SUMMARY

The aldehydic crosslink precursors and their crosslinks from sodium borotritiide reduced dermal collagens of normal human skin and Ehlers-Danlos Syndrome skin were compared. The two reduced compounds dominating the elution profile in Ehlers-Danlos Syndrome are hydroxynorleucine and a new basic compound eluting after lysine. This latter predominant peak has rarely been witnessed in a reduced collagen. Relatively small amounts of hydroxylysinonorleucine and "post histidine" peak are present. The elution profile is much less complex in nature than dermal collagen from normal human skin. The results suggest that Ehlers-Danlos Syndrome may be a collagen crosslink disorder.

INTRODUCTION

Investigations into the intermolecular aldehydic crosslink precursors and their Schiff base crosslinks in collagen have made rapid progress. It has been demonstrated that intermolecular covalent crosslinks form when tropocollagens in solution are reconstituted to fibrils (1,2). More complex patterns of reducible precursors and crosslinks are present in the insoluble soft tissue collagens (3) while those of mineralized tissues are simpler in nature (4).

It was shown earlier than α-amino-adipic-δ-semialdehyde (1), α-amino-δ-hydroxy-adipic-δ-semialdehyde (2), and the intramolecular covalent crosslink, aldol condensation product (5), partake in crosslink formation. It was later reported that as aldol condensation product disappears in reconstituting fibrils from tropocollagen, a "post histidine" peak appears (6). The reduced crosslinks lysinonorleucine (7) and dihydroxylysinonorleucine (3) have been isolated from insoluble collagen fibrils. Hydroxylysinonorleucine

has been isolated from reconstituted fibrils (8) and also from insoluble collagen (9). The compound "syndesinol" was isolated from reduced dentinal collagen by Bailey et al. (10) and was misidentified by the latter authors as the reduced mixed aldol condensation product between α-amino-adipic-δ-semialdehyde and α-amino-δ-hydroxy-adipic-δ-semialdehyde. The true identity of the compound was established as dihydroxylysinonorleucine by Mechanic et al. (4) and was also shown to exist as an actual crosslink because it was partially reduced in vivo. Following the latter report Davis and Bailey (11) reported on their re-evaluation of the structure of "syndesinol" and confirmed the structure previously described by Mechanic and Tanzer (3) as dihydroxylysinonorleucine.

In the heritable connective tissue disorder Ehlers-Danlos Syndrome, the basic defect is thought to concern the lack of organization of the collagen bundles into an intermeshing network (12). The joints as well as the skin are hyperextensible, yet not lax. It has been reported that the ultramicroscopic appearance of collagen and elastin are quite normal (15). Shrinkage temperatures of Ehlers-Danlos Syndrome tendon collagen does not differ from normal tendon collagen (16). Biochemical abnormalities, such as increased turnover of collagen and differences in plasma elastase inhibitor, were sought and could not be found (17). It was proposed that although the quantity of collagen and elastin is normal (13), the abnormality was due to defective binding of the collagen fibrils which are linked together to make up the collagen bundles. Another similar proposal was put forth by Strelling (14) where he suggested that the fibrils were able to slide over each other more easily because of abnormal formation of collagen bundles. In other words, it seems as though the macromolecular matrix of collagen lacks some stabilizing forces. Barker and Kennedy (19) proposed that since dermal incorporation into Ehlers-Danlos skin of chondroitin 4-sulphate was low while that of chondroitin-6-sulphate was correspondingly high, the syndrome was a mucopolysaccharide disorder.

This paper reports the lack of reducible intermolecular crosslinks in dermal collagen of Ehlers-Danlos Syndrome.

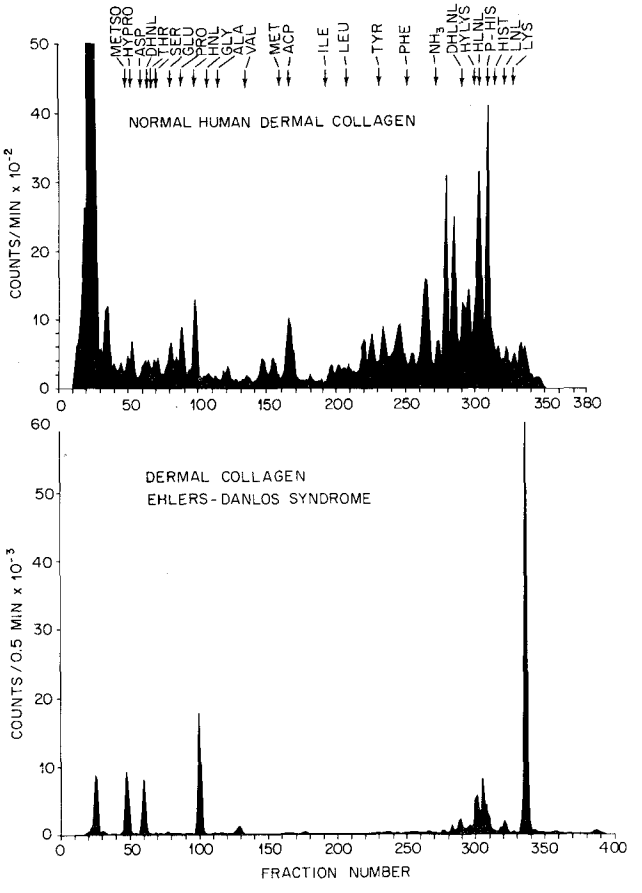
METHODS

Dermal collagen obtained from normal human skin and from human Ehlers-Danlos Syndrome skin was minced and reduced with the same specific activity sodium borotritiide as described (1). The samples were hydrolyzed with 3N tosyl acid (20) for 24 hours; aliquots representing 6.5 mg and 6.0 mg respectively were chromatographed using an amino acid analyzer column as described (1).

RESULTS

The elution profiles of the radioactive components from normal human and Ehlers-Danlos Syndrome dermal collagens are shown in Figure 1 and the peaks are identified in the legend. In the collagen from Ehlers-Danlos Syndrome the major peak that dominates the pattern is a new reducible compound that elutes after lysine. This peak is about 3.5 times greater than hydroxynorleucine which is the second largest peak in the pattern. The two peaks eluting just prior to dihydroxynorleucine are unknown and may represent reduced aldehydic compounds not observed previously. Hydroxylysinonorleucine is a minor component as well as is the "post histidine" peak. Dihydroxylysinonorleucine is all but absent as well as lysinonorleucine. No peak corresponding to reduced aldol condensation product is seen. Reduced elastin was run as a control and none of the peaks except for hydroxynorleucine correspond to the profile in Fig. 1 of Ehlers-Danlos Syndrome. Reduced aldol condensation product in elastin served to identify its position in the chromatogram.

In the normal skin collagen a complete complement of reducible intermolecular crosslinks is observable. The "post histidine" peak is the predominant crosslink. A large amount of hydroxylysinonorleucine is present. The ratio of these are reversed in this pattern. The two peaks eluting just after



LEGEND TO FIGURE 1

Chromatographic fractionation of 3N tosyl acid hydrolyzates of NaB^3H_4 reduced human dermal collagens. Upper figure is that of normal skin and lower is that of Ehlers-Danlos Syndrome. The order of elution is 64-67, dihydroxynorleucine; 97-103, hydroxynorleucine; 163-169, reduced self aldol condensation product of α-amino-adipic-δ-semialdehyde; 300-306, hydroxylysinonorleucine; 307-311, "post histidine" peak; 320-323, lysinonorleucine; 333-340, previously unknown reducible compound.

NH_3 are also presentin considerable abundance. Note the hydroxynorleucine is considerably smaller than the crosslink peaks. The peak just after Met corresponds to reduced aldol condensation product.

DISCUSSION

The results indicate clearly that major differences exist in the extent and nature of the crosslinks present in the two collagens. The fact that

there is a large amount of hydroxynorleucine and only a small amount of hydroxylysinonorleucine evident in the pattern from Ehlers-Danlos Syndrome indicates that α-amino-adipic-δ-semialdehyde has not been utilized effectively in forming Schiff base type crosslinks. The presence of the two radioactive compounds appearing prior to that of hydroxynorleucine may indicate that other unidentified reducible aldehydes exist in collagen and just have not been utilized here. The "post histidine" peak is seen as a very minor component and may indicate that the intramolecular crosslink aldol condensation product is virtually non-existent in the dermal collagen of Ehlers-Danlos Syndrome since a precursor-crosslink relationship has been shown to exist between the two (6). There also is no peak indicating aldol condensation product while one exists in the pattern obtained from normal skin collagen. The extremely large radioactive peak eluting after lysine is as yet unidentified and has no explanation at this time.

From the evidence presented here it is suggested that the Ehlers-Danlos Syndrome may be a heritable collagen crosslink disorder that exists on a molecular level.

The fact that collagen from Ehlers-Danlos Syndrome forms normal ultramicroscopic fibrils (15) and its shrinkage temperature is normal (16) indicates that the collagen aggregates properly into fibrils. Scanning electron microscopy has also indicated that the fibre bundles are of irregular orientation (18). It has been observed that the micromolecular structure of an uncrosslinked polypeptide chain of a protein may contain all the "information" necessary for assembly of a native structure (21,22, 23). This has been attributed to as being a result of specific interactions formed between amino acid residues lying along the chain in the required sequence. A large amount of hydroxynorleucine does not react to form the normal complement of intra- and intermolecular crosslinks. This suggests that micromolecular aberrations might exist in the collagen molecule, and therefore the molecules are not brought into correct juxtaposition for the

normally reactive precursor residues to form crosslinks. As a result the macromolecular collagen bundles lack the stabilization forces needed to produce a normal cohesive supporting matrix.

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