COLLAGEN CROSSLINKS: OCCURRENCE IN BASEMENT MEMBRANE COLLAGENS

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SUMMARY

Basement membrane preparations from anterior lens capsule, Descemet's membrane, and renal glomerulus were reduced with NaB^3H_4 in order to label carbonyl-derived crosslinks. Quantitative incorporation of tritium into the basement membranes was found, similar to the levels observed in fibrous collagens. A considerable proportion of the label was chromatographically identical with the reduced aldehydes and crosslinks of collagen, suggesting that it is the collagenous portions of basement membrane which contain these compounds. This was substantiated by showing that the isolated collagenous proteins contained substantial amounts of reduced aldehydes and crosslinks.

The collagenous proteins in higher animals can be categorized into two classes, the interstitial and basement membrane collagens (1,2). The interstitial collagens constitute the bulk of the total collagen, occurring as characteristic extracellular fibres in such tissues as bone, skin, tendon and blood vessels. Basement membranes are also extracellular, are associated with vascular endothelium and epithelial linings of all body tissues, are morphologically amorphous and have unique properties and compositions. Basement membrane collagens are unusually rich in hydroxylysine and carbohydrate and are genetically distinct (2,3). However, the fundamental polypeptide unit, the α chain, is present in both collagens, as are the characteristic amino acids hydroxyproline, hydroxylysine, the glycosylhydroxylysine units and glycine, which accounts for one-third of the amino acid residues (3,4). Thus, there are several biochemical features common to both types of collagen, suggesting that they may have other similarities. In this report we describe

the presence of reducible aldehydes and aldehyde-derived crosslinks in the basement membrane collagens of lens capsule, renal glomerulus and Descemet's membrane. These reducible compounds are chromatographically identical with known compounds found in interstitial collagens.

EXPERIMENTAL

Basement membranes were isolated from bovine and sheep anterior lens capsule, from canine and human renal glomerulus and from lamb Descemet's membrane by previously described methods (4,5). Reduction of disulfides and alkylation of sulphydryl groups was carried out, as was urea extraction, citrate extraction and limited proteolysis by pepsin (4,5,7). The various preparations were lyophilized and stored in dry form. Representative samples were rehydrated in K^+ $PO_4^{\equiv}$, pH 7.6, ionic strength 0.15 and were treated with NaB^3H_4 as described (8). The treated material was thoroughly washed free of reagents by distilled water and was then lyophilized. Hydrolysis in 3 N HCl was done, followed by ion exchange chromatography. Bovine skin "insoluble" collagen was reduced by NaB^3H_4 in the same way and was used as the interstitial collagen standard.

RESULTS

The incorporation of tritium from NaB^3H_4 was measured in acid hydrolysates of the various preparations (Table I). The amount of tritium taken up by the basement membrane samples varied from .62 m μmoles/mg to 3.59 m μmoles/mg, a range which corresponds to the amount found for cow skin collagen (Table I). These values necessarily are minimal figures because certain substances are destroyed by acid hydrolysis (10).

Chromatographic fractionation of the hydrolysates showed that a number of the radioactive substances appeared in the area of known reduced aldehydes and crosslinks (Fig. 1). Other radioactive substances, whose identities are not established, were also present. The reduced aldehydes, hydroxynorleucine and dihydroxynorleucine were present in substantial quantity, accounting for up to

TABLE I

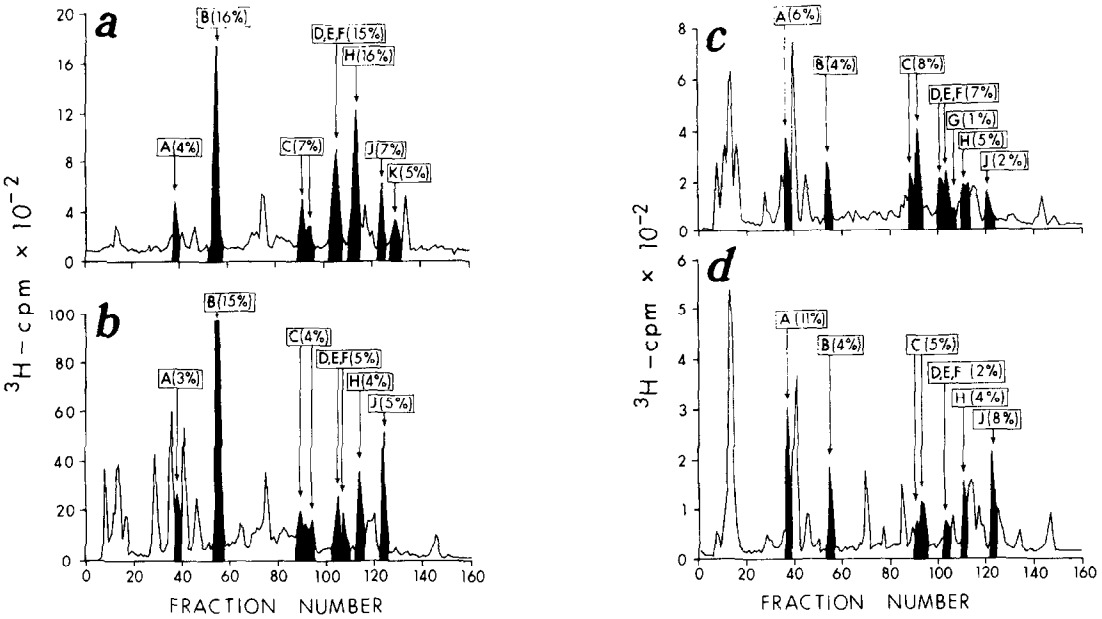
Tritium content of acid hydrolysates of NaB^3H_4-reduced basement membranes and basement membrane collagens

Preparation	cpm/mg† x 10^{-5}	m μmoles ^3H*/mg
Bovine skin insuluble collagen	1.20	2.91
Sheep anterior lens capsule	0.52	1.28
Lamb Descemet's membrane	0.90	2.19
Dog glomerular basement membrane; reduced and alkylated in 8 M urea	1.12	2.71
Human glomerular basement membrane; residue after 8 M urea extraction	0.69	1.67
Bovine anterior lens capsule collagen; citrate extract	0.26	0.62
Bovine anterior lens capsule collagen; pepsin isolation	0.62	1.50
Bovine anterior lens capsule; reduced and alkylated in 8 M urea	1.48	3.59

† mg of dry weight prior to hydrolysis.

* m μmoles ^3H calculated by standardizing (9) the NaB^3H_4 preparation which was used for all reductions.

20% of the radioactivity eluted from the column. Similarly, a large proportion of radioactivity was present in the region from fractions 85-150, where all of the crosslinks elute. Thus, the crosslinks N^{ε}-hexosyl hydroxylysine, aldol-histidine, dihydroxylysinonorleucine, hydroxylysinonorleucine, lysinonorleucine, and hydroxymerodesmosine were present in the chromatograms, but the



1. Ion exchange chromatography of selected acid hydrolysates from Table I. Conditions described in (8). The samples were: A) Bovine skin insoluble collagen; B) Lamb Descemet's membrane; C) Sheep anterior lens capsule; D) Bovine anterior lens capsule; citrate extract. The other samples in Table I were also subjected to chromatography and yielded similar results (see text). The peaks are: A, dihydroxynorleucine; B, hydroxynorleucine; C, N^{ε}-hexosylhydroxylysines; D, N^{ε}-hexosyllysines; E, aldolhistidine; F, dihydroxylysinonorleucine; G, hydroxymerodesmosine; H, hydroxylysinonorleucine; J, lysinonorleucine; K, histidinohydroxymerodesmosines. The percentage of the total eluted radioactivity represented by each peak is noted in parentheses in the figure.

histidino-hydroxymerodesmosines did not appear in the chromatograms of the basement membrane collagens. As noted in the case of interstitial collagens, the relative abundance of a particular compound varied with the individual collagen preparation, reflecting such parameters as species, tissue characteristics, or the age of the animal (11).

The origin of the collagen-related radioactive peaks could be attributed to the collagenous regions of the basement membrane because the peaks were present in purified collagens obtained from the basement membranes. Thus, citrate extracted collagen from bovine anterior lens capsule had a chromatographic profile similar to that of cow skin collagen (compare Fig. 1A and 1D). Furthermore, the collagen released by limited pepsin digestion of the lens

capsule had a chromatographic profile similar to Fig. 1A and 1D. In contrast, the total basement membrane preparations (Fig. 1B and 1C) had more complex chromatographic profiles due to the presence of additional radioactive peaks whose identity is unknown. Results similar to those of Fig. 1B and 1C were found for the total basement membrane from the glomerulus (Table I), whereas the urea-residue fraction of the human glomerulus contained relatively small amounts of radioactivity eluting in fractions 85-150.

The new radioactive peaks in the chromatograms of the basement membranes were generally in the region where neutral and acidic amino acids elute (fractions 10-80), suggesting that these new compounds were not of the general structure found for collagen crosslinks. The preparations which had been reduced and alkylated prior to NaB^3H_4 exposure had very high amounts of radioactivity early in the chromatogram, in fractions 10-20. The other prominent peaks often occurred adjacent to dihydroxynorleucine, at fractions 34-36 and 40-42 (Fig. 1).

DISCUSSION

The results show that: 1) basement membrane preparations incorporate tritium during NaB^3H_4 reduction in amounts similar to interstitial collagens; 2) a large proportion of the radioactivity is present in peaks which correspond to reduced aldehydes and crosslinks; 3) it is the collagenous proteins of the basement membranes which contain the reduced aldehydes and crosslinks. Thus, interstitial and basement membrane collagens have an additional common biochemical feature, the presence of aldehydes and aldehyde-derived crosslinks. In view of their other common biochemical properties, it is not altogether surprising that the two types of collagen should contain similar aldehydes and crosslinks. The marked insolubility of basement membrane collagens may in part be explained by the presence of these compounds although other components such as disulfide crosslinks also play a major role (2).

Presumably the aldehydes in both types of collagen arise by the same pathway, via the enzymatic action of lysyl oxidase (12). Crosslinking in the

interstitial collagens occurs after fibril formation, and a high degree of specificity appears to be important (13,14). Whether a similar sort of specificity occurs in basement membrane collagens cannot be ascertained at present; the absence of the crosslink histidino-hydroxymerodesmosine in Fig. 1B, C, D suggests that a different degree of specificity is operative since this particular crosslink is derived from histidine, hydroxylysine and 2 allysines, all of which are present in basement membranes.

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