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THREE NEW α-CHAINS OF COLLAGEN FROM A NON-BASEMENT MEMBRANE SOURCE

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Summary: Three new collagen α chains have been isolated from synovial membrane, gingiva and skin. Two of these have a similar chromatographic behaviour to the α[A] and α[B] chains described by Burgeson et al. (4) from a foetal basement membrane source but have been separated from another contaminating α chain, α[C]. The α[A] and α[B] chains are present in approximately equal amounts. They contain no detectable 3-hydroxyproline, are highly glycosylated and all sugar residues are present as the disaccharides. The percentage of hydroxylation of the lysine is of the order of 70%. Only a third of these hydroxylysine residues are glycosylated. The significance of these peptides, present in a tissue substantially free of basement membrane, is discussed.

At the present time four different genetic types of collagen have been characterised (1). With the exception of Type I collagen which has two α_1 chains and one α_2 chain all contain three identical α chains. Types II, III and IV collagen are classically isolated by pepsin digestion of their tissue source followed by differential salt precipitation of the solubilised products. Recently attention has been turning towards minor components which are also extracted by pepsin but which had previously been overlooked. These components are very soluble in quite high salt concentrations (3.2M). Several descriptions of such components have appeared but they have all been prepared from tissues with a high basement membrane content and it has generally been assumed that they are either basement membrane collagens or components of basement membranes (2,3). Burgeson and his colleagues (4) described two new collagen α chains from human foetal basement membranes

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which they called A and B chains and suggested from the proportions of the two α chains that the new collagen was $\alpha A\alpha[B]_2$.

We report here the presence of collagen α chains similar to these A and B chains but which do not contain 3-hydroxyproline, an amino acid present in basement membrane collagen. These chains have been isolated from synovial membrane, a collagenous tissue which does not include a basement membrane (5) and they appear to be present in the same proportion in normal and inflamed tissue which suggests that they are not of vascular origin. The isolated collagen chains are highly glycosylated and appear to be nearer to a 1 : 1 than a 1 : 2 ratio. A third component was found which is also collagenous and runs between the A and B chain peaks on elution from carboxymethyl cellulose columns and is closely associated with the α[B] type chain on SDS-polyacrylamide gel electrophoresis.

MATERIALS AND METHODS

Synovia were obtained from surgical synovectomy or post-mortem dissection. Collagen molecules were released from the tissue by pepsin digestion and separated by differential salt precipitation as described (6). Amino acid analysis was performed as previously described (7). Carbohydrate residues were analysed after methanolysis as their silane derivatives on a Pye gas-liquid chromatograph. We are grateful to Dr. David Milsom for performing the GLC analysis.

Electrophoresis on SDS-polyacrylamide gels (5%) both with and without mercaptoethanol reduction was performed either by the method of Furthmayr and Timpl (8) or by the discontinuous system of Laemmli (9).

Carboxymethyl-cellulose chromatography was according to (10). Periodic acid Schiff reagent (PAS) was used to locate carbohydrate containing bands on electrophoretic gels (11). Clostridiopeptidase B was obtained from Boehringer Corporation (London) Ltd. and contained less than 3% non-specific protease activity (J. Anderson, personal communication).

RESULTS AND DISCUSSION

Pepsin solubilised collagen was fractionated by salt precipitation at neutral pH. Collagen fractions were obtained at 0.86M, 1.5M and 2.6M NaCl.

The supernatant was then examined by SDS polyacrylamide gel electrophoresis. One principle PAS positive band with an apparent M.W. of 125,000 daltons together with a fainter, also PAS positive band running in the same position as α_1[I] were seen. The principle band has an electrophoretic mobility similar to the α[B] band of Burgeson et al. (4).

Eight synovial membranes (two normal and six from rheumatoid or non-rheumatoid inflamed joints), a foetal calf skin, a bovine placentum, an inflamed canine gingiva and a bullock tendon were all examined for the presence of these PAS positive bands in the 2.6M NaCl supernatant. They were found to be present in all tissues with the exception of bullock tendon. The amount of collagen present in the 2.6M NaCl supernatant as a percentage of the total pepsin solubilised collagen was between 1% and 2% of the total in all synovial tissues. The solubility of these components in relatively high salt concentrations suggested that they might be present in neutral salt and acid extracts of the non-pepsin treated tissue and these were examined but no PAS positive bands were found. This confirms that the material is only released after pepsin digestion.

The 2.6M NaCl supernatant was made to 3.2M with NaCl and a precipitate was obtained (PPT1). The supernatant was dialysed into 4.0M NaCl and a further precipitate obtained (PPT2). These two precipitates accounted for approximately 0.7% and 1.2% of the total pepsin solubilised collagen respectively. By utilisation of a discontinuous system of polyacrylamide gel electrophoresis the α[B] band could be separated into two PAS positive bands, one remaining at the original 125,000 M.W. position and one having a slightly greater electrophoretic mobility (Fig. 1). Thus three PAS positive bands can be seen. We have called them α[A], α[B] and α[C]. In PPT2 these bands are virtually free from contamination with Type I collagen. The α[A] band which runs in the same position as α[I] gave a PAS positive reaction; in our hands α_1[I] itself never gives a positive reaction with PAS.

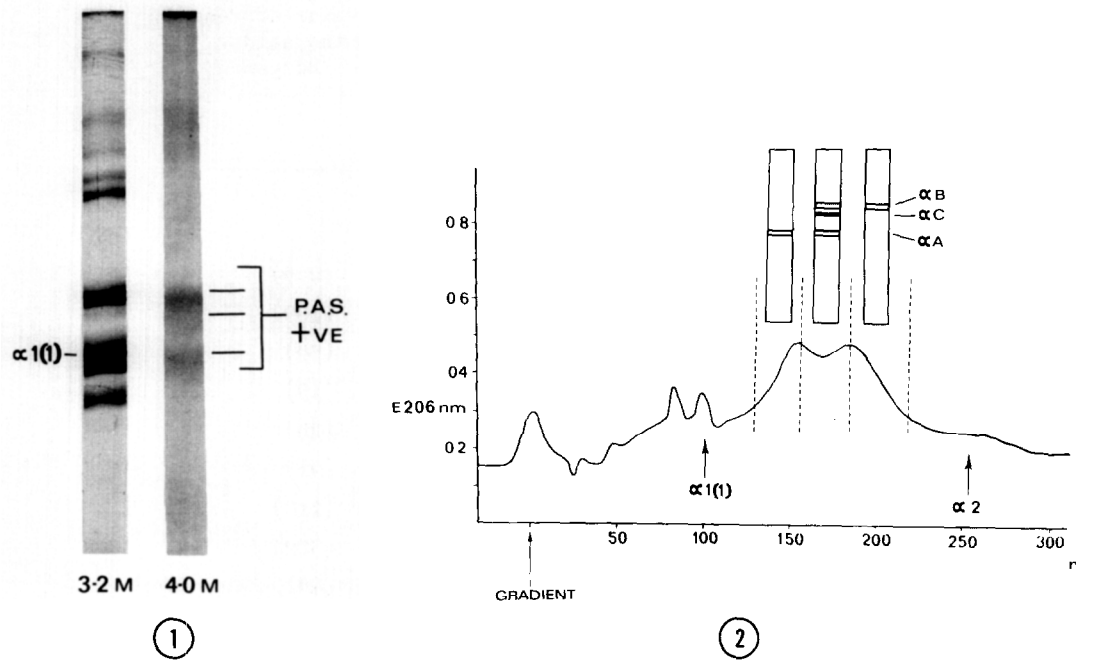

Fig. 1.

Polyacrylamide gel electrophoresis on SDS using a discontinuous buffer system (9) of PPT1 (3.2M NaCl) and PPT2 (4.0M NaCl). Bands were located with both Coomassie Blue and Periodic acid-Schiff reagent.

Fig. 2.

a) Elution pattern from a carboxymethyl-cellulose column (12 cm x 1.5 cm) of the 4.0M NaCl precipitate (PPT2). (Elution gradient 0 - 0.1M NaCl over 400 ml). The bars indicate which samples were taken for amino acid analysis.

b) Electrophoresis in a discontinuous buffer system on polyacrylamide gel with SDS shows that the samples taken for amino acid analysis are pure.

After digestion with clostridiopeptidase B all bands disappeared, suggesting that they are collagenous. No change was observed in the αA and αB chains after further pepsin digestion and reduction with β mercapto-ethanol.

The α[A] and α[B] bands were separated from α[C] and from one another by carboxymethyl cellulose chromatography of PPT2 (Fig. 2). Polyacrylamide gel electrophoresis showed that the α[A] and α[B] chains were free from each

Table 1. Amino acid analysis of α[A] and α[B] chains expressed as amino acid residues per 1000 total amino acids. The figures in brackets refer to those of Burgeson et al. (4).

	αA		αB	
3OH Pro*	-	(2.5)	-	(2.9)
4OH Pro	117.0	(109)	119.5	(109)
Asp	53	(51)	43	(50)
Thr	28	(26)	18	(19)
Ser	43	(31)	23	(26)
Glu	99	(84)	99	(91)
Pro	113.7	(97)	139.5	(118)
Gly	314	(319)	341	(322)
Ala	51	(52)	46	(46)
Cys	-	-	-	-
Val	31	(27)	26	(18)
Met	10	(11)	6	(8)
Ile	13	(16)	15	(19)
Leu	30	(35)	34	(39)
Tyr	trace	(1.8)	trace	(2.1)
Phe	13	(14)	14	(12)
HyLys	27.3	(24)	30.2	(35)
Lys	13.2	(18)	10.7	(20)
His	7	(11)	3	(7.5)
Arg	37	(68)	31	(50)

* In our analytical system we would expect to detect 3-OH proline if present in this amount.

other and from contaminating α[C] chains. Amino acid analyses are shown in Table I. The principle differences between the two α chains were in the numbers of proline, serine and threonine residues. In both chains the percentage hydroxylation of the lysine was very high, 67% for α[A] and 74% for α[B], the percentage hydroxylation of proline being different for the two chains. Gas liquid chromatography showed that glucose and galactose

were present in equimolar amounts, suggesting that these sugars were present only as the disaccharides. The analyses also indicated that only about a third of the hydroxylysine residues were glycosylated. It may be that the others are involved in reducible cross-linking.

The question arises as to the origin of these new collagens. They are either relatively minor components or they are in high concentration in the insoluble residue which does not respond to pepsin degradation. The only tissue which we found did not contain them was bullock tendon. Tendon is a wholly Type I collagen and it may be that the new collagens are present only in association with Type III collagen. It is possible to speculate that the new α chains derive from capillaries or small blood vessels present in the tissues. However we did not find appreciably more of the new material in whole placenta where, of course, there are very large numbers of capillary vessels, nor was there a greater proportion in inflamed than in non-inflamed synovial membranes.

The presence of three different α chains suggests that there are three more genes coding for collagen than has previously been assumed. Burgeson and his co-workers (4) concluded that the α[B] and α[A] chains from foetal membranes were present in a 2 : 1 ratio. The α[A] and α[B] collagens we have isolated are present in roughly equal amounts. The organisation of three new α chains must necessarily be speculative but two situations could be envisaged - an $\alpha[A]_3$, $\alpha[B]_3$ and $\alpha[C]_3$ or a shared α[C] chain with either $(\alpha[A])_2$ or $(\alpha[B])_2$. Either configuration would suggest at least two new collagen species.

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