TYPE V COLLAGEN: POSSIBLE SHARED IDENTITY OF αA, αB and αC CHAINS

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1. Introduction

Collagen is known to exist in tissue in a variety of polymorphic forms. Four genetically distinct types have so far been acceptably defined [1]. Type I collagen is found alone in bone and tendon and type II collagen is also found on its own in cartilage. Both are found together in the intervertebral disc. Type III collagen has so far been found in tissue only in association with type I, mixed polymers of type I and type III collagen are present in skin, blood vessels and other elastic tissues and they probably make up the bulk of the connective tissue collagen in the body.

Recently, a new form of collagen has been described, called variously AB collagen and type V collagen [2–7]. A common source of this new collagen is the placentum although it has also been identified in extracts of calfskin, diseased and normal synovial membrane, aorta and cartilage. There are at present two suggested molecular structures for AB collagen. The first postulates that two peptide chains form one molecule, $[\alpha A(\alpha B)_2]$ [3–5] which has been called type V, whereas the second view is of two molecules $(\alpha A)_3$ and $(\alpha B)_3$ [6]. A third peptide chain (αC) [7] has so far not been allocated a position in either formula. Work in this laboratory supports the concept of two separate molecules consisting of either three αA or three αB chains and proposes a possible third molecule, $(\alpha C)_3$. We further suggest that all are modifications of the same basic molecule.

2. Materials and methods

Synovial membranes were obtained at synovectomy and stored in sterile dishes at −20°C before extraction. Foetal calfskin was obtained freshly from the abattoirs. The tissues were defatted manually before being pulverised in a stainless-steel bomb with liquid N_2. The powder was extracted in three changes of acetone to remove lipids then washed exhaustively in 0.5 M NaCl to remove soluble proteins. The insoluble residue was spun down at 300 × *g* for 20 min at 4°C and exhaustively extracted with 0.2 M acetic acid. Insoluble material was again removed by centrifugation and resuspended in a solution of pepsin in 0.2 M acetic acid to give an *E* : *S* 1 : 10. The mixture was incubated at 18°C for 5 days with the addition of further pepsin (*E* : *S* 1 : 20) on day 3 and pepsin solubilised collagen was removed by centrifugation at 18 000 × *g* for 1 h at 4°C the supernatant was filtered where necessary and the dissolved collagen precipitated by dialysis into 0.02 M Na_2HPO_4. Most of the pepsin remained in solution. The precipitate was redissolved in 0.2 M acetic acid and dialysed into 0.86 M NaCl in 50 mM Tris–HCl (pH 7.5) until an equilibrium pH was obtained. Any precipitate was removed by centrifugation. Types III and I collagen were precipitated by further dialysis into 1.5 M and 2.6 M NaCl in buffer. A further dialysis into 3.2 M NaCl precipitated contaminating type I collagen and the AB collagen was precipitated relatively pure, at 4.0 M NaCl (pH 7.5). This precipitate was redissolved in 0.2 M acetic acid and frozen at −20°C.

Peptide chains of collagen were denatured and chromatographed on CM-cellulose with a linear gradient from 0–0.1 M NaCl over 500 ml total vol. as in [8].

Cyanogen bromide digestion was essentially by the method [9]. Cyanogen bromide fragments and intact collagen peptides were run on vertical slab apparatus using 10% and 5% gels, respectively, as in

[10], though without a spacer gel. Peptide bands were located with Coomassie blue R 250 (Biorad; Richmond, CA), 1.25% in 20% trichloroacetic acid for 30 min and destained in 7% acetic acid : 14% methanol. Glycosylated peptides were located with periodic acid Schiff's (PAS) reagent (by immersion in 1% periodic acid solution, in 3% acetic acid for 30 min, followed by washing overnight in a large volume of distilled water and reacting with Schiff's reagent (Hopkin and Williams, Romford) for 30 min. Densitometric scans, at 580 nm, were made of individual tracks from the slab using an SP 8-100 Spectrophotometer (Pye-Unicam, Cambridge) with gel scanning attachment.

3. Results

Precipitation of native (A,B,C) collagen at 4.0 M NaCl with prior removal of type I at 2.6 M and 3.2 M NaCl, successively, was found to be a very efficient purification on the basis of SDS–polyacrylamide gel electrophoresis. Figure 1 shows the collagen patterns obtained from two such precipitates.

Clearly, there is very little α2 collagen present in the 4.0 M precipitate. PAS treatment of such gels allowed more positive identification of the bands since type I collagen chains have never given positive PAS staining in our hands even when gels have been heavily loaded. αA, αB and αC bands on the other hand are strongly positive. A minor, unidentified band ran slightly ahead of the αC band (fig.1, III).

Separation of the collagen chains was achieved by CM-cellulose chromatography (fig.2). The two major peaks eluted in a similar position to αA and αB chains from placentum [3]. Fractions under the bars 1 and 2 were pooled and analysed by polyacrylamide gel electrophoresis. Despite the bias from the centre of the peaks, fraction 1 was found to contain components which corresponded to the αA and αC chains present in the original 4.0 M precipitate. The second peak fraction 2, contained only αB chains.

The amino acid composition was consistent with published values for AB collagen [3,5,7]. L-3-hydroxyproline was not present in detectable amounts but valine and hydroxylysine were high and alanine was relatively low (table 1).

Cyanogen bromide peptide patterns were obtained from the αB chain and the αC plus αA chain mixture

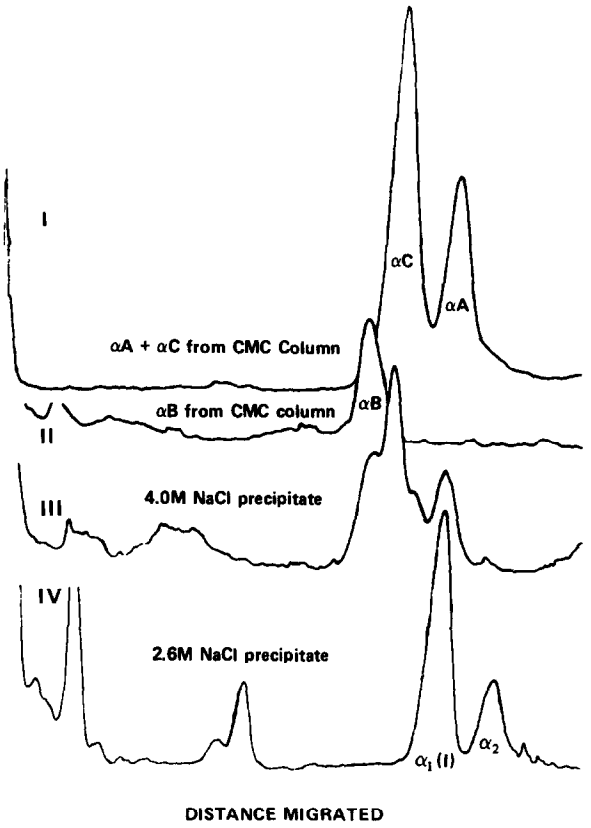

Fig.1. Densitometric scans of SDS–gel (5%) electrophoresis separated α chains from bovine skin I, αA + αC from fraction 1 (see fig.2); II, αB from fraction 2 (see fig.2); III, total 4.0 M NaCl precipitate; IV, total 2.6 M NaCl precipitate.

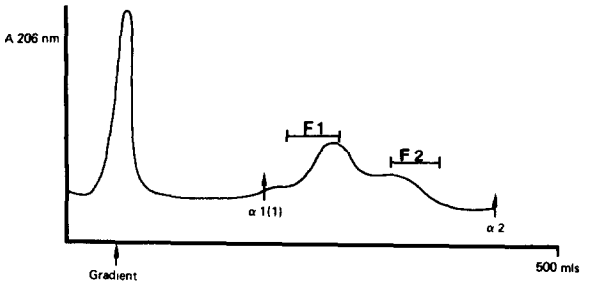

Fig.2. Separation pattern on CM-cellulose of the 4.0 M NaCl precipitate from calfskin (0–0.1 M NaCl gradient over 500 ml sodium acetate buffer (pH 4.8)).

Table 1
Comparison of amino acid analysis of new collagen α chains from various sources

Bovine skin		Human synovia		Amino acid
αA + αC	αB	αA	αB	
142	85	117	119	Hyp
48	47	53	43	Asp
25	24	28	18	Thr
31	40	43	23	Ser
97	109	99	99	Glu
117	162	114	140	Pro
332	350	314	341	Gly
52	62	51	46	Ala
–	Tr	–	–	Cys
18	13	31	26	Val
6	16	10	6	Met
11	11	13	15	Ile
27	28	30	34	Leu
Tr	Tr	Tr	Tr	Tyr
8	6	13	14	Phe
31	31	27	30	Hyl
15	16	13	11	Lys
Tr	Tr	7	3	His
41	37	37	31	Arg
55%	34%	51%	54%	% Pro/OH
68%	66%	67%	74%	% Lys/OH

(foetal calfskin) and from αA and αB chains of inflamed synovial membrane.

The molecular weights of the separated components were estimated using CNBr peptides of known molecular weights from α1 (I), α2 and α1 (II) collagen chains as standards. Densitometric traces showed no similarity with α1 (I), α2, α1 (II) or α1 (III) chains CNBr peptides but the αA and the αB chains (or the α(A + C) and αB chains in the case of skin) did show many similar features. The striking similarity was between the patterns from αB and the mixed αA and αC chains from foetal calfskin shown in fig.3. Two high molecular weight bands, no. 3,7 had a reduced intensity in the α(A + C) chain patterns. However, the most clearly modified region was in the central area, peaks 9 and 10, where a component, number 9, in the α(A + C) mixture, seemed to run slightly faster (app. mol. wt 35 500 in the mixture and 34 000 in the αB chain). This may reflect a decrease in molecular weight of a single peptide, alternately it is possible that the peak in question contains more than one component.

A similar picture emerged from the CNBr digestion of human synovial membrane αA and αB chains. A comparison of the patterns obtained from isolated chains strongly suggests that they are fundamentally the same (fig.4). One feature of the patterns from synovial αA and αB chains was the different proportion of high and low molecular weight bands.

In particular there was a greater proportion of low molecular weight material in the αA pattern than in the αB. A 1 : 1 mixture of αA and αB chains gave a more balanced pattern reflecting a compromise between the two components. Since many of the higher molecular weight peptides probably contain intact methionine residues [11,12] it may be that the cleavage of the αA chain from this tissue was more complete than the αB. However, it must be said that the amounts of AB collagen from synovia are small and it was therefore difficult to obtain such clear densiometric traces as we have for skin. Careful examination reveals that all of the major high molecular weight peaks in the synovial αB chain are present in the αA chain and that the difference is quantitative not qualitative. One minor peak in the high molecular weight region of the synovial αB chain is apparently absent from the αA chain and vice versa. These minor differences are far outweighed by the overall similarities of the two patterns.

PAS location of glycosylated peptides in the CNBr pattern gave only two positive bands. One ran with the tracker dye and the second corresponded to band no. 14 (fig.3) seen on Coomassie blue staining. It would seem likely that the glycosidic bonds are labile to the formic acid treatment used in the CNBr cleavage.

4. Discussion

Although the presence and appearance of at least two of the peptides of AB collagen (namely αA and αB) are now well known their interaction with one another and with the intermediate sized αC chain is not understood. It has been suggested [3,5] that the collagen molecule of this species is composed of two αB and one αA chain [αA $(αB)_2$]. This is supported by the relative peak sizes of these two peptides on CM-cellulose chromatography of bovine placental extracts and on physico-chemical studies during renaturation [5].

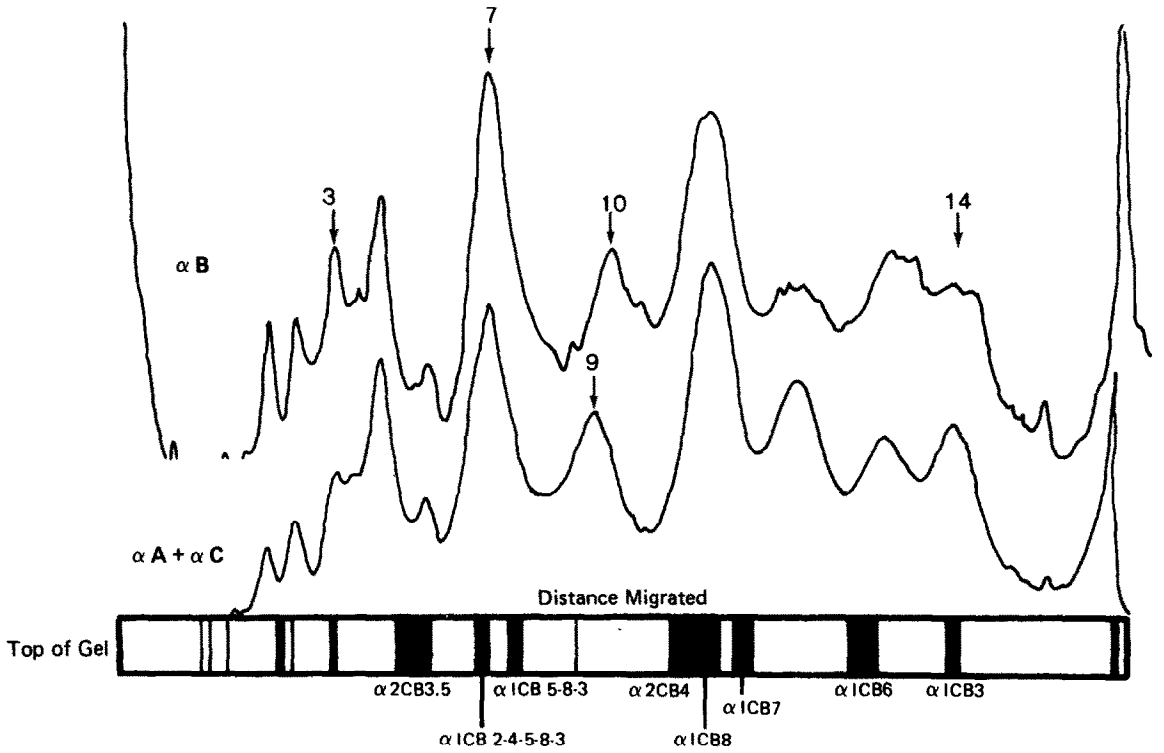

Fig.3. Densitometric scans of cyanogen bromide peptide patterns from separated αB chain and mixed αA + αC chains of bovine skin (10% gel). The equivalent pattern of type I collagen is shown at the bottom for comparison. Numbered peaks are referred to in the text.

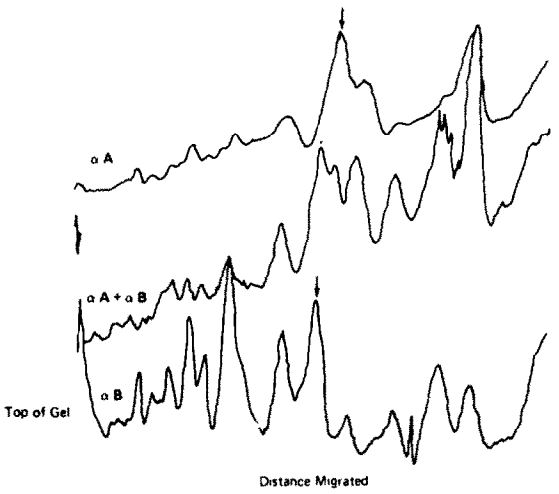

Fig.4. Densitometric scans of cyanogen bromide peptide patterns from separated αA and αB chains and mixed αA + αB chains from human synovial membrane (10% gel). Arrowed peaks indicate the main differences.

The alternative theory of their association as $(\alpha B)_3$ $(\alpha A)_3$ molecules has been argued on the basis of ratios other than 2 : 1 for B and A chains from other tissues [6] and on denaturation studies. We have failed to find ratio of 2 : 1 for B : A chains in either synovial membrane or skin. In skin another chain, αC, was the major component, appearing to increase the αA peak with which it co-chromatographed. Extracts were made on two occasions from two portions of synovial membrane from the same patient with juvenile ankylosing spondilytis, the first extraction gave a 1 : 1 ratio for A and B (with only traces of αC) [7] but the second extraction gave only a single peak on CM-cellulose in the position of αB (R.A.B., J.B.W., unpublished data).

These apparently puzzling features of type AB collagen chain ratios are probably explained by the CNBr peptide patterns which show profound similarities between the αA and the αB chains from synovial membrane and also the αB and a mixture of the αA

and αC chains of skin. These findings are consistent with the idea of a common ancestoral peptide chain for the αB, αA and αC components. Modification of the original molecule is most probably by proteolytic cleavage at specific sites giving rise to progressively smaller molecules. In this respect the appearance of yet another potential member of this club is of interest. The peptide can be seen in fig.1, just ahead of the αC chain. We tentatively propose the term αD for this chain!

One of the most striking features of the CNBr patterns from the calfskin AB collagen peptides is the clarity and high resolution of the bands. This was not the case for other tissue sources, for example, synovial membrane, which gave a pattern for αA with a large proportion of low molecular weight bands and some streaking. Conversely, a preponderance of high molecular weight material was found in the αB chain pattern. Nevertheless the similarities between the overall patterns of AB collagens from the two tissue sources is worth noting. This is particularly significant since the quantities of available starting material were different in both cases.

The relative contribution of the αC band to the αA + αC pattern of skin is not possible to determine but the pattern cannot represent one chain alone based on the proportions of the two chains present in the starting material – 1.7 : 1 (fig.1, I). In any case it is worth pointing out that all the high molecular weight bands in αB are in fact present in αA in the synovial collagen, albeit in much reduced amounts. Differences in the extent of hydroxylation of lysine residues in αA and αB chains and in the extent of their glycosylation have been reported [3,7] and such differences may be tissue specific. The amino acid analyses of αB chains from skin and synovia gave virtually the same number of hydroxylysine residues, and a similar level of hydroxylation for all chains.

It is possible, then by using the hypothesis of shared identity, to explain most of the conflicting features found in the study of AB collagen and also to explain how the component chains are just sufficiently different from one another to separate on SDS–polyacrylamide gel electrophoresis and ion exchange chromatography, and even to have slight differences in amino acid analysis and CNBr peptide pattern. We are of the opinion that type V collagen is made up of three single identical parent α chains but that methods for their preparation, which involve extensive proteolysis, may be causing artifactual modifications. However, we cannot rule out the possibility that these modifications could occur in the tissues, prior to extraction – the nature of the intact precursor must await less drastic methods of preparation.

Acknowledgements

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