IDENTIFICATION OF A NEW DISULPHIDE BONDED COLLAGEN FROM CARTILAGE

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1. Introduction

Type II is the predominant collagen found in hyaline cartilage [1,2] but a small proportion of 3 additional polypeptide chains have been isolated and partially characterised. The composition of two of the new components, 1α and 2α was reported [3] to resemble the αA and αB chains of type V [4], whilst the third chain, 3α, was shown to be similar to type α(II) minor prepared from bovine cartilage [5]. The molecular composition of these 3 chains is unknown, and micro-analysis of the cartilage failed to reveal a spatial distribution of these new collagen types. Although several other types of fibrous collagen have been reported in various tissues [6], and more recently some disulphide bonded molecules in basement membrane collagen [7–9], none of these has been identified in cartilage. This communication reports the isolation and partial characterisation of a novel collagen type from neonatal pig hyaline cartilage which, unlike the 1α, 2α and 3α components [3], is reduced to smaller fragments by mercaptoethanol, indicating that the chain is stabilised by intra-chain disulphide bonds.

2. Materials and methods

2.1. *Pig hyaline cartilage collagen preparation*

Cartilage tissues from neonatal pig were prepared as in [10]. Essentially, thin tissue slices were homogenised and the proteoglycans extracted in 4.0 M guanidine chloride. After centrifugation and washing with distilled water, the pellet was suspended in 0.5 M acetic acid and digested with pepsin at a substrate: enzyme ratio of 10:1 at 4°C for 24 h. The solubilised collagens were then separated by salt fractionation as in [10] with a slight modification. After precipitation of collagen at 0.7 M NaCl and 1.2 M NaCl consecutively at pH 2.5, NaCl was increased to 2.0 M when a further precipitate was obtained.

2.2. *Pig placenta collagen preparation*

The total tissue was treated as in [7,11] and the pepsin digestion carried out at 16°C for 6 h. The solubilised collagens were then subjected to the thermal gelation technique [12] to remove the interstitial type I and type III collagens. After dialysing against 0.5 N acetic acid, the remaining collagen was submitted to salt fractionation [10]. From the acidic solution the 0.7 M NaCl precipitate contained the types I and III collagen; the 1.2 M NaCl precipitate, type V collagen, and the 2.0 M NaCl fraction contained a high molecular weight collagen. The latter was further purified by solubilising in 1.0 M NaCl, 0.05 M Tris (pH 7.4) and solid NaCl added to give 4.5 M NaCl final conc. All precipitates were subsequently dialysed extensively against dilute acetic acid and lyophilised.

2.3. *Methods*

SDS–polyacrylamide gel analysis of the polypeptide chains was done using a flat-bed apparatus and employing Tris-borate buffers (pH 7.4) as in [13].

Where applicable the lyophilised collagen was reduced with dithiothreitol and alkylated with iodoacetamide as in [7].

Prior to chromatography on CM-cellulose columns, samples were passed down a DEAE-cellulose column equilibrated with 0.2 M NaCl, 0.05 M Tris, 2.0 M urea, (pH 7.4) in order to remove contaminating acidic glycoproteins. CM-cellulose chromatography was done in 0.04 M sodium acetate buffer, at 42°C and pH 4.8 with a 0.012–0.12 M in 400 ml NaCl gradient total vol. For amino acid analysis, samples

were hydrolysed at 110°C in 6 N HCl for 24 h and analysed on a Jeol 6AH amino acid analyser.

3. Results

SDS-polyacrylamide gel electrophoretograms of the purified fractions from hyaline cartilage are shown in fig.1. Fig.1b,c shows that the 0.7 M NaCl precipitate contains solely type II collagen. The 1.2 M NaCl fraction shows at least 3 major bands with a lower mobility than that of the α_1(II) chain, all of which are unaffected by the reducing agent, mercaptoethanol (fig.1d,e). Chromatography of the 1.2 M NaCl fraction on a CM-cellulose column separated the 3 polypeptides (fig.2a), their elution positions being consistent with those in [3] for 1α, 2α and 3α.

The 2.0 M NaCl fraction also shows several bands (fig.1f,g), but the major component has a mobility slightly less than α_1(II) chain. However, this α-chain is reducible with 2-mercaptoethanol to give a band with an app. M_r ~33 000. Additional bands are apparent in the 2.0 M NaCl fraction from adult tissue.

The 2.0 M NaCl fraction before reduction appeared as a broad peak on a CM-cellulose column (fig.2b) which when further analysed by SDS–polyacrylamide gel electrophoresis gave a single component of app. M_r 110 000. Reduction and alkylation of this 2.0 M NaCl fraction produced two peaks on the CM-cellulose column, one of which was not retained by the column, whilst the other was eluted just prior to the known position of α_1(I) (fig.2c). This latter peak was shown by SDS–polyacrylamide gel electrophoresis to be the 33 000 M_r component. The other rapidly eluting component has not yet been further characterised. The amino acid composition of the 110 000 and 33 000 M_r chains eluted from the CM-cellulose column were found to be virtually identical and rather similar to basement membrane collagen (table 1).

In addition to comparing the new reducible 110 000 M_r chain with type II, 1α, 2α and 3α from cartilage, it was also compared with type V and 7S collagen isolated from porcine placenta. The solubilised collagens from placental tissue gave distinctly different solubility properties, electrophoretic patterns and CM-cellulose column chromatograms. Type V (B_2A) was precipitated from acid solution at 1.2 M NaCl and possessed different electrophoretic mobilities. The fraction precipitating at 2 M NaCl was redu-

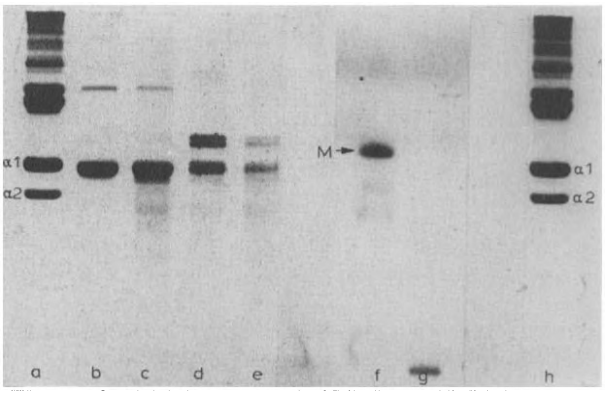

Fig.1. SDS–polyacrylamide gel electrophoresis pattern of collagens solubilised by pepsin treatment of porcine articular cartilage: (a) rat-tail tendon collagen; (b,c) 0.7 M NaCl precipitate without and with mercaptoethanol, respectively; (d,e) 1.2 M NaCl precipitate without and with mercaptoethanol, respectively; (f,g) 2.0 M NaCl precipitate without and with mercaptoethanol, respectively; (h) rat-tail tendon collagen.

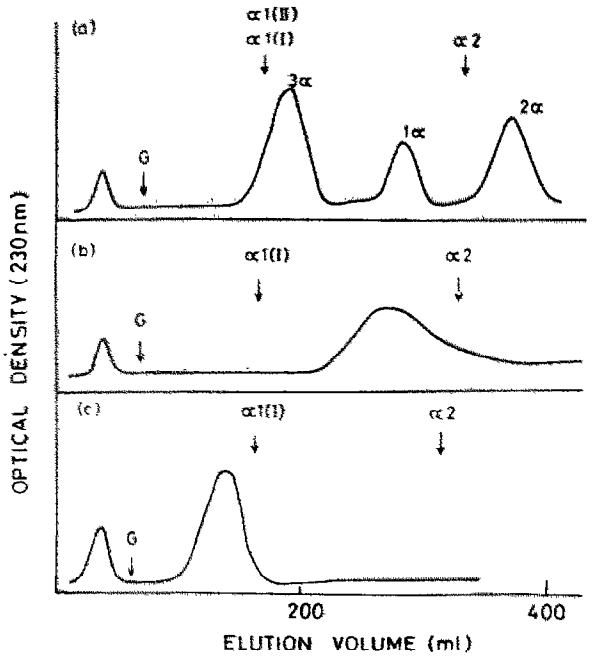

Fig.2. Carboxymethyl-cellulose column chromatograph of collagen isolated from porcine articular cartilage after pepsin treatment. The location of the α1 and α2 chains of type I collagen are shown for reference: (a) 1.2 M NaCl precipitate showing the resolution of the 1α, 2α and 3α chains; (b) 2.0 M NaCl precipitate; (c) 2.0 M NaCl precipitate reduced and alkylated prior to chromatography.

Table 1
Amino acid composition of type M collagen

	Type M	Type IV [7]	Type II [15]	Type 7S [9]
3 Hyp	–	7.0	–	3
4 Hyp	90.0	121.0	92	115
Asp	39.0	55.8	44	50
Thr	19.6	25.3	21	26
Ser	27.5	36.1	25	29
Glu	114.2	100.1	94	91
Pro	93.6	81.6	114	77
Gly	339.4	290.6	336	326
Ala	56.6	41.5	105	25
Cys	–	5.6	–	18
Val	35.6	27.5	16	30
Met	–	9.9	9	12
Ile	20.8	29.0	9	19
Leu	44.2	49.8	28	49
Tyr	4.0	9.6	2	10
Phe	5.8	26.8	13	25
Hyl	34.2	36.3	21	46
Lys	19.1	14.6	16	7
His	6.7	5.1	3	8
Arg	49.3	32.1	52	34

cible with mercaptoethanol to several polypeptide chains consistent with the patterns reported for 7S collagen in [8,9]. The components of the new collagen isolated from cartilage were clearly distinct from these basement membrane-type collagens (fig.3).

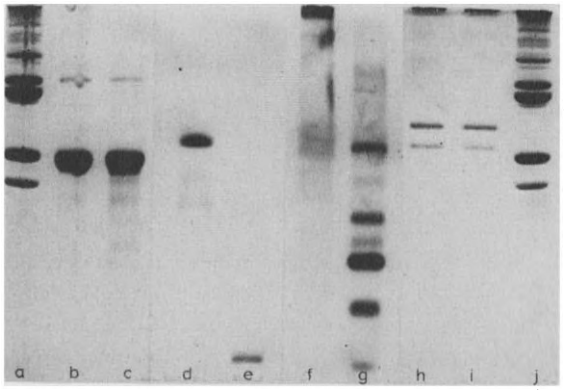

Fig.3. SDS–polyacrylamide gel electrophoresis pattern of the 2.0 M NaCl precipitate of collagen isolated from articular cartilage compared with type 7S and type V collagen isolated from porcine placental tissue: (a) rat-tail tendon; (b,c) type II collagen from cartilage; (d,e) 2.0 M NaCl precipitate from cartilage without and with mercaptoethanol, respectively; (f,g) type 7S collagen from placenta without and with mercaptoethanol, respectively; (h,i) type V collagen from placenta without and with mercaptoethanol, respectively; (j) rat-tail tendon.

4. Discussion

A new type of collagen comprised of 3 identical α-chains of app. M_r 110 000, with the property of being reduced to 33 000 M_r components, has been identified in cartilage. This new collagen constitutes ~5–10% of the total collagen and it is more soluble than type II collagen, precipitating at 2.0 M NaCl compared to 0.7 M NaCl for the latter, and 1.2 M NaCl for the minor components 1α, 2α and 3α identified in [3]. The precipitation of the collagen from solution clearly indicates the presence of native collagen molecules, and the identification of a single α-type chain suggests a molecular composition of 3 identical α-chains. We have provisionally designated this collagen type M collagen.

Careful comparison of the properties of type M collagen with the known cartilage collagens, the predominant type II collagen and the minor 1α, 2α and 3α has shown it to possess distinctly unique properties, particularly in respect of its sensitivity to reducing agents.

Further comparisons with other collagens identified from tissues other than cartilage have been carried out. Type V (B_2A) collagen [4], type IV [7] and the 7S collagen identified [8], and similarly failed to reveal an identity with these collagens. Although not

isolated in these studies, the collagen identified [14] from placental membranes capable of reduction to 40 000 M_r components is unlike type M in that it exists as a high molecular weight aggregate. We are therefore convinced that type M collagen is a genetically distinct new type of collagen.

Type M was initially found in neonatal cartilage but its presence was subsequently demonstrated in adult articular and bronchial cartilage. This fact and the relatively high proportion (5–10%) of type M in cartilage argues against it originating from the fetal cartilage vascular system. However, its basic similarity to the other disulphide stabilised collagen [8,14] found in other tissues as components of basement membrane may be significant. Future studies on the location of this new collagen by immunofluorescent staining should throw some light on the distribution and possibly the function of type M collagen in cartilage.

Acknowledgements

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