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Minor Collagens of Chicken Hyaline Cartilage†

Charles A. Reese and Richard Mayne*

ABSTRACT: Analysis has been made of the minor collagens which are solubilized by limited pepsin digestion of chicken sterna and which remain in solution after precipitation of type II collagen at 0.9 M NaCl–0.5 M HOAc. The precipitate obtained by further dialysis to 1.2 M NaCl–0.5 M HOAc was shown to contain the 1α, 2α, and 3α chains previously isolated from human cartilages [Burgeson, R. E., & Hollister, D. W. (1979) *Biochem. Biophys. Res. Commun. 87*, 1124–1131]. Mapping by polyacrylamide gel electrophoresis of the CNBr and *Staphylococcus aureus* V8 protease peptides derived from the 1α, 2α, and 3α chains strongly suggested that (i) 1α and 2α are different from the α1(V) and α2(V) chains of type V collagen and (ii) 3α is closely related to the α1(II) chain. An additional collagenous molecule of higher molecular weight (called HMW) was present in the 1.2 M NaCl precipitate, and considerably more HMW could be precipitated by dialysis to 2.0 M NaCl–0.5 M HOAc. HMW contained disulfide bonds and, after reduction, gave rise to three components, C-1, C-2, and C-3, of apparent M_r 87 500, 51 000, and 36 400, respectively. The precipitate obtained at 2.0 M NaCl–0.5 M HOAc also contained a lower molecular weight collagenous molecule (called LMW), which contained disulfide bonds and had an apparent molecular weight before reduction of 30 000. Cultured chick chondrocytes isolated from embryonic sterna were shown to synthesize 1α, 2α, and 3α by radioactive labeling and fluorography of polyacrylamide gels. The amino acid analyses and solubility properites of 1α, 2α, and HMW demonstrate that these components are closely related to the type V collagen isolated from other tissues, and it is suggested that 1α, 2α, and HMW should be regarded as members of a type V family of collagens.

Several studies have shown that the major collagen present in all hyaline cartilages is type II collagen of chain composition [α1(II)]$_3$ [reviewed by Miller (1976)]. However, several groups have recently reported the isolation of small amounts of other collagenous molecules from hyaline cartilages. Burgeson & Hollister (1979), working with human and bovine hyaline cartilages, isolated three collagenous chains which were designated 1α, 2α, and 3α. These authors proposed that 1α and 2α were previously undescribed collagen chains but were nevertheless closely related to the α1(V) and α2(V) chains of type V collagen.[1] The chain designated 3α appeared closely related to the α1(II) chain of type II collagen and may be an overglycosylated form of this chain. These results have been confirmed for neonatal pig hyaline cartilage (Shimokomaki et al., 1980) and for bovine nasal cartilage (Ayad et al., 1981), with both of these groups also reporting the isolation of an additional collagenous component of apparent M_r 110 000, which contained disulfide bonds and was reducible to a single component of apparent M_r 33 000.

In this paper, we report our analyses of the minor collagens present in chicken hyaline cartilage. The 1α, 2α, and 3α chains have been isolated from this tissue, and in addition, two disulfide-bonded collagenous molecules have been obtained which are similar to, but nevertheless are different from, the disulfide-bonded molecule previously isolated from mammalian cartilages (Shimokomaki et al., 1980; Ayad et al., 1981).

Materials and Methods

Isolation and Fractionation of Collagens. Sterna (200 g) from adult chickens were stripped of perichondrium, diced into cold, distilled water, and homogenized by using a Polytron homogenizer (Brinkman Instruments). After centrifugation, the sterna were extracted with 4 M guanidine, 50 mM Tris-HCl,[2] pH 7.4 (two extractions, 12 h each, 4 °C), followed by extensive washing of the sterna with cold, distilled water. The sterna were resuspended in 0.5 M acetic acid and 0.2 M NaCl containing pepsin (1 mg/mL, Worthington) as described previously (Burgeson & Hollister, 1979) and extracted with gentle stirring (4 °C, 20 h). After centrifugation (30000*g*, 30 min), the supernatant was brought to pH 8.0 by addition of 5 M NaOH. After standing overnight, the solution was

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[1] The nomenclature used for the type V collagen chains is that proposed by Bornstein & Sage (1980), so that the previously described αB and αA chains (Burgeson et al., 1976) are now designated the α1(V) and α2(V) chains.

[2] Abbreviations used: CNBr, cyanogen bromide; CM, carboxymethyl; $NaDodSO_4$, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane.

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dialyzed extensively against 0.9 M NaCl in 0.5 M acetic acid (four changes, 96 h), and the precipitate of type II collagen was removed by centrifugation. The supernatant was then dialyzed against 1.2 M NaCl in 0.5 M acetic acid (four changes, 96 h), and the resulting precipitate was also recovered by centrifugation. Finally, a precipitate was obtained by dialysis against 2.0 M NaCl–0.5 M acetic acid (four changes, 96 h). All precipitates were dissolved in 0.5 M acetic acid, followed by extensive dialysis against 0.1 M acetic acid and lyophilization. Standard preparations of chicken types I, III, and V collagen were isolated from adult chicken gizzards as described in previous publications (Mayne & Zettergren, 1980; Mayne et al., 1980).

Polyacrylamide Gel Electrophoresis. Initial characterization of collagenous fractions obtained after differential salt precipitation was carried out by $NaDodSO_4$–polyacrylamide gel electrophoresis using 5–7.5% gradient slab gels. The conditions of electrophoresis were as described by Laemmli (1970), except for the inclusion of urea (8 M) in the gel. Peptides obtained after CNBr or *S. aureus* V8 protease digestion (see below) were fractionated by using 9–15% gradient gels without the inclusion of urea in the gel. Staining and destaining of the gels were carried out as described previously (Mayne & Zettergren, 1980).

CM-cellulose Chromatography. Samples obtained after differential salt precipitation or molecular sieve chromatography were fractionated by CM-cellulose chromatography (Whatman, CM-32) under denaturing conditions as described previously (Mayne & Zettergren, 1980).

Molecular Sieve Chromatography. The precipitate obtained at 2.0 M NaCl/0.5 M HOAc was denatured (55 °C, 30 min) and fractionated by chromatography on a column (2.5 × 155 cm) of agarose beads (Bio-Gel A-5m, 200–400 mesh) with elution conditions as described previously (Mayne & Zettergren, 1980). Calibration of the column was achieved with a mixture of chicken gizzard type I and type III collagens which contained γ, β, and α components.

Amino Acid Analyses. Samples were dissolved in 6 N HCl and hydrolyzed at 105 °C for 20 h in the atmosphere of N_2. Separation of amino acids was achieved with a Beckman 121C amino acid analyzer and an elution program of buffers for a single column as described previously (Mayne & Zettergren, 1980).

Peptide Mapping after CNBr Cleavage. Samples were dissolved in 70% formic acid (1 mg/mL) and incubated with CNBr (10 mg/mL) in an atmosphere of nitrogen for 4 h at 30 °C. The sample solution was diluted with water, lyophilized, and analyzed by polyacrylamide gel electrophoresis.

Peptide Mapping after S. aureus V8 Protease Cleavage. Samples were dissolved at a concentration of 2 mg/mL in 0.125 M Tris-HCl, pH 6.8, containing 10% sucrose and 0.005% bromophenol blue and then denatured (100 °C, 30 s). *S. aureus* V8 protease (Miles) was dissolved in the same solution (1 mg/mL), and digestion was carried out with an enzyme to substrate ratio of 1:20 (30 min, 37 °C). At the end of the incubation, $NaDodSO_4$ was added to a final concentration of 0.1%, and after heating (100 °C, 30 s), the samples were applied to a polyacrylamide slab gel (9–15% gradient gel).

Biosynthesis of the Minor Collagens by Cultured Chick Chondrocytes. Chondrocytes were isolated from the sterna of 13-day-old chick embryos and selected as "floaters" as described previously (Schiltz et al., 1973). The cells were lightly trypsinized (0.2% trypsin, 20 min), plated into tissue culture dishes (100 mm, Falcon), and grown for 5 days in Ham's medium F-10 plus 10% fetal calf serum. Cultures were

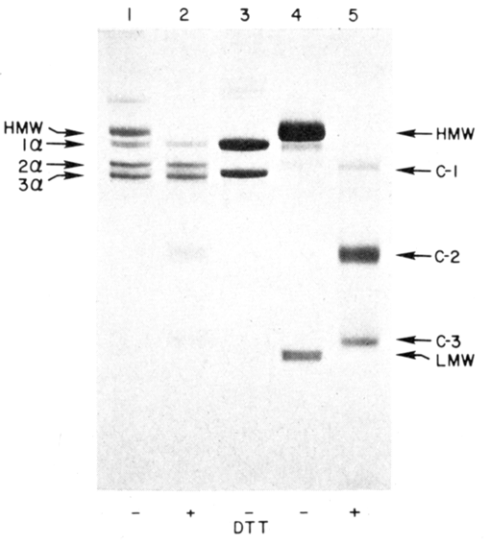

FIGURE 1: $NaDodSO_4$–polyacrylamide gel electrophoresis (5–7.5% gradient gel) of fractions obtained after differential salt precipitation either with or without reduction (50 mM dithiothreitol, 100 °C, 1 min). The samples (10–20 μg of protein) were (lanes 1 and 2) the precipitate at 1.2 M NaCl–0.5 M HOAc, (lane 3) type V collagen from chicken gizzard, and (lanes 4 and 5) the precipitate at 2.0 M NaCl–0.5 M HOAc.

labeled for 24 h with complete medium containing [2-^{3}H]-glycine (50 μCi/mL, 15.0 Ci/mmol, New England Nuclear) in the presence of β-aminopropionitrile (100 μg/mL) and ascorbic acid (50 μg/mL). The cell layers were scraped into the medium, and ammonium sulfate was added (25% w/v). After standing overnight at 4 °C, the pellet obtained after centrifugation was incubated for 6 h at 4 °C with pepsin (200 μg/mL, Sigma) in 0.5 M HOAc (total volume 15 mL) in the presence of carrier collagen. The carrier consisted of the precipitates obtained from a pepsin digest of adult chicken cartilage at 0.9 M NaCl–0.5 M HOAc and 1.2 M NaCl–0.5 M HOAc (5 mg each). After incubation, pepsin was inactivated by dialysis against 0.5 M NaCl and 0.05 M Tris-HCl, pH 8.0 (three changes), and the undigested material was removed by centrifugation. Ammonium sulfate (25% w/v) was added to the supernatant, and after standing at 4 °C for 24 h, the precipitate of collagen was removed by centrifugation. The pellet was resuspended in 0.5 M HOAc (10 mL), and precipitates were obtained by differential salt precipitation at 0.9 M NaCl–0.5 M HOAc and 1.2 M NaCl–0.5 M HOAc. Each precipitate was dissolved in 0.5 M HOAc, desalted by dialysis against 0.1 M HOAc, lyophilized, and analyzed by polyacrylamide gel electrophoresis.

Fluorography of Polyacrylamide Gels. After electrophoresis, gels were placed in EN^{3}HANCE (3 volumes, 1 h, New England Nuclear) and gently shaken. The gels were washed with deionized water (1 h), dried down, and exposed for varying times to X-Omat-R X-ray film (Kodak) at −70 °C.

Radioactivity Determinations. Aliquots (0.5 mL) of the fractions obtained during CM-cellulose chromatography were mixed with 5 mL of Scintiverse (Fisher) for determination in a liquid scintillation counter (Model LS 7000, Beckman Instruments).

Results

Figure 1 shows an analysis by polyacrylamide gel electrophoresis of the precipitates obtained after dialysis of the pepsin digest to 1.2 M NaCl–0.5 M HOAc and subsequently after dialysis to 2.0 M NaCl–0.5 M HOAc. Three prominent bands were observed in the 1.2 M NaCl precipitate, which were unaffected by reduction, and correspond to the expected

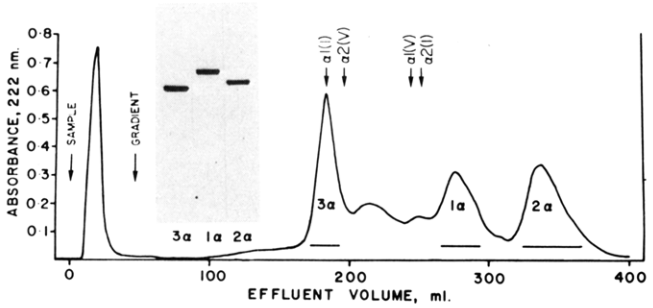

FIGURE 2: CM-cellulose chromatography of the precipitate obtained at 1.2 M NaCl–0.5 M HOAc. The column (1.5 × 10 cm) was equilibrated at 42 °C with 0.02 M (Na^+) sodium acetate, pH 4.8, containing 4 M urea, and elution was achieved with a linear gradient of 0.0–0.12 M NaCl at a flow rate of 100 mL/h over a total volume of 400 mL. Arrows show the elution positions of the α1(I) and α2(I) chains of type I collagen and the α1(V) and α2(V) chains of type V collagen. Bars show the three fractions which were pooled for further analysis. The inset shows polyacrylamide gel electrophoresis of these fractions (5–7.5% gradient gel, without reduction).

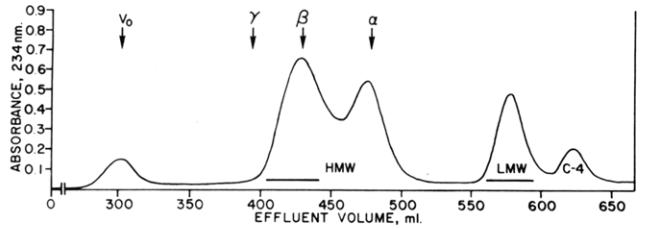

FIGURE 3: Agarose (Bio-Gel A-5m) molecular sieve elution pattern of the precipitate obtained after dialysis against 2.0 M NaCl–0.5 M HOAc. The column (2.5 × 155 cm) was eluted with 1 M $CaCl_2$ in 50 mM Tris-HCl, pH 7.5, at a constant flow rate, and the sample (40 mg) was denatured in 6 mL of this solution. Arrows show the elution positions of γ (M_r 285 000), β (190 000), and α (95 000) components after calibration of the column with a mixture of chicken gizzard type I and type III collagens. Bars indicate the fractions which were pooled for further analysis.

migration positions of the 1α, 2α, and 3α chains (lanes 1 and 2). A fourth band was also observed which migrated more slowly than 1α and was no longer present after reduction. We call this latter material, which is collagenous by amino acid composition (see below), the high molecular weight fraction or HMW. Considerably more HMW was present in the 2.0 M NaCl precipitates (lane 4), and after reduction, HMW gave rise to three collagenous components which we call C-1, C-2, and C-3 (lane 5). The 2.0 M precipitate also contained a lower molecular weight fraction (called LMW) which, after reduction, gave rise to two components which were no longer retained in the gel.

Further characterization of the 1.2 M NaCl–0.5 M HOAc precipitate was achieved by CM-cellulose chromatography in denaturing conditions. Figure 2 shows the separation of several components by this procedure, the three most prominent peaks corresponding to the expected elution positions for the 3α, 1α, and 2α chains. Material present in each of the three peaks was desalted, lyophilized, and shown to consist of a single chain by polyacrylamide gel electrophoresis (Figure 2, inset). The material which eluted between 3α and 1α was also examined by polyacrylamide gel electrophoresis and found to consist almost entirely of HMW. We have not been able to demonstrate the presence of the α1(V) and α2(V) chains in chicken hyaline cartilage, thus confirming the results of Shimokomaki et al. (1980) and Ayad et al. (1981) for mammalian cartilages and contrary to a report that human hyaline cartilage contains exclusively the α1(V) chain (Rhodes & Miller, 1978), which from immunofluorescence staining has been localized to the pericellular matrix (Gay et al., 1981).

The precipitate obtained at 2.0 M NaCl–0.5 M HOAc was denatured and fractionated by molecular sieve chromatography (Figure 3). A prominent peak was observed which eluted in the region of β chains and was subsequently shown to be HMW by polyacrylamide gel electrophoresis. However, part of the HMW fraction also eluted in the region of α chains. We have been unable to demonstrate any difference in the behavior of HMW isolated from the two peaks on polyacrylamide gel electrophoresis either with or without reduction. If the material present in either peak was denatured and rechromatographed, it again showed a separation into two peaks during agarose gel filtration. The reason for this behavior is at present unknown and may relate to the formation of dimers of some of the molecules of HMW during denaturation, which remain together during agarose gel filtration. LMW was also eluted from the column, followed by a small fraction we call C-4. Recently, we have shown that C-4 is a small non-disulfide-bonded collagenous fragment present in native molecules of HMW and derived from HMW during denaturation (C. A. Reese and R. Mayne, unpublished observations). Further fractionation and purification of HMW was achieved by CM-cellulose chromatography (Figure 4) in which HMW was observed to elute as a single, broad peak between the location of the α2(V) and α1(V) chains. HMW migrated as a single band on polyacrylamide gel electrophoresis (Figure 4, inset) which, with reduction, gave rise to the three components C-1, C-2, and C-3.

Amino acid compositions of HMW, LMW, 1α, 2α, α1(V), α2(V), 3α, and α1(II) are shown in Table I. Comparison of the analyses for 3α and α1(II) shows very close similarities

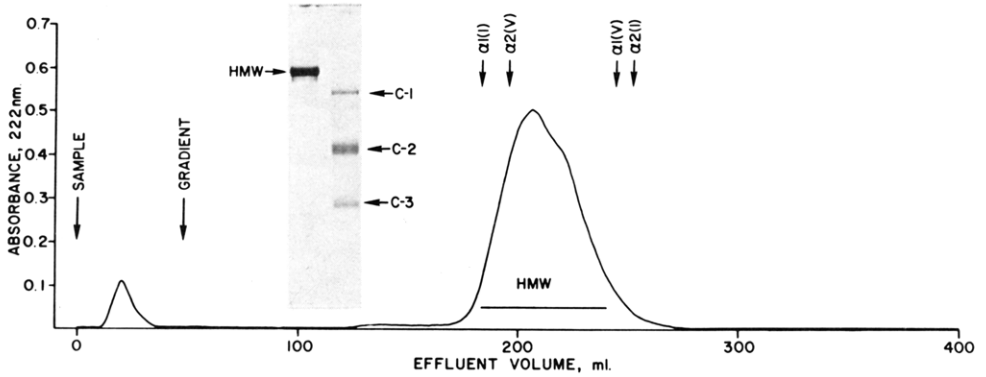

FIGURE 4: CM-cellulose chromatography of the high molecular weight fraction (HMW) after initial separation by agarose gel chromatography (Figure 3). Chromatography was performed as described in Figure 2. The bar indicates the fractions which were pooled for further analysis. Inset shows polyacrylamide gel electrophoresis of HMW (5–7.5% gradient gel) with and without reduction (50 mM dithiothreitol, 100 °C, 1 min) after initial fractionation by CM-cellulose chromatography.

Table I: Amino Acid Compositions of the Components Recovered after Agarose Gel or CM-cellulose Chromatography[a]

amino acid	residues/1000							
	HMW	LMW	1α	2α	α1(V)	α2(V)	3α	α1(II)
3-Hyp					7	5		2
4-Hyp	95	105	98	96	111	116	118	103
Asp	53	50	48	46	45	47	44	42
Thr	16	20	13	14	16	23	19	26
Ser	38	22	37	34	26	38	26	26
Glu	97	81	105	91	102	92	90	87
Pro	90	92	117	110	114	104	111	115
Gly	323	310	321	336	335	306	322	329
Ala	57	68	63	57	45	67	112	104
½-Cys[b]	2	9						
Val	23	25	22	31	17	26	18	16
Met[c]	9	11	6	7	7	14	5	11
Ile	27	24	17	13	19	16	8	8
Leu	48	53	38	33	41	36	26	26
Tyr	5	6	4	4	2	1	2	2
Phe	8	8	12	13	11	11	14	15
Hyl	34	20	33	35	41	22	19	23
Lys	21	20	18	17	14	14	13	13
His	8	10	7	14	5	6	2	2
Arg	46	66	41	49	42	56	51	50

[a] Each analysis is expressed as residues/1000. LMW was analyzed after recovery from agarose gel filtration; all other components were analyzed after recovery from CM-cellulose chromatography. No corrections were made for loss of threonine or serine or the incomplete release of valine. [b] Determined as *S*-(carboxymethyl)cysteine. [c] Determined as the sum of methionine and methionine sulfoxide.

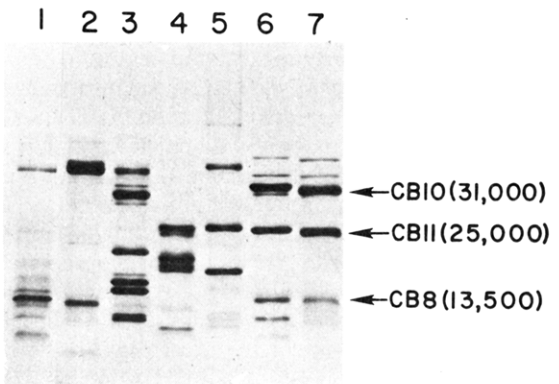

FIGURE 5: $NaDodSO_4$–polyacrylamide gel electrophoresis (9–15% gradient gel) of peptides obtained after CNBr cleavage of samples. The cyanogen bromide cleavage reaction was performed in 70% formic acid with a 10:1 ratio by weight of CNBr to sample. Arrows show the migration positions and molecular weights of peptides obtained after cyanogen bromide cleavage of α1(II) chains. Lanes were (1) HMW, (2), 1α, (3) 2α, (4) α2(V), (5) α1(V), (6) 3α, and (7) α1(II). Each lane was loaded with 50 μg of sample.

and, together with the peptide mapping results (see below), strongly suggests that 3α is an overglycosylated form of the α1(II) chain as suggested previously (Burgeson & Hollister, 1979). Analyses of 1α, 2α, α1(V), and α2(V) were all similar to each other and typical for type V collagen with a relatively low alanine content when compared to the interstitial collagens. The analyses for HMW and LMW were also typical for type V collagen, except for the presence of cystine residues.

The various samples were cleaved by CNBr and the resulting peptides analyzed by polyacrylamide gel electrophoresis (Figure 5). Comparison of the banding patterns obtained for 3α and α1(II) (lanes 6 and 7) showed very strong similarities, with the peptides derived from 3α migrating slightly more slowly than those derived from α1(II). This behavior is similar to that previously described by Burgeson & Hollister (1979) for 3α and α1(II) chains isolated from human cartilage. The

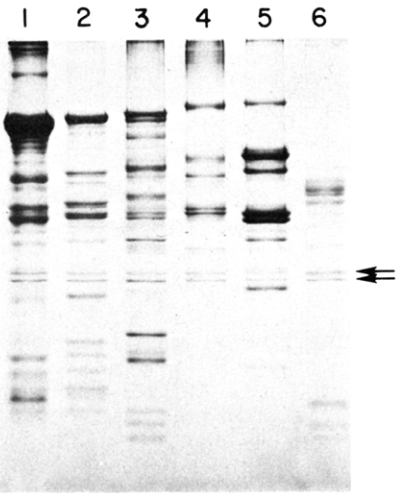

FIGURE 6: $NaDodSO_4$–polyacrylamide gel electrophoresis (9–15% gradient gel) of peptides obtained after *S. aureus* V8 protease digestion. The digestion was performed in electrophoresis sample buffer, but without $NaDodSO_4$ (37 °C, 30 min, enzyme to substrate ratio 1:20). Before electrophoresis, $NaDodSO_4$ (final concentration 0.1%) was added and the sample heated (100 °C, 30 s). Lanes were (1) α1(II), (2) 3α, (3) 2α, (4) 1α, (5) α1(V), and (6) α2(V). Each lane was loaded with 50 μg of sample. Arrows indicate the doublet band of the V8 protease enzyme.

peptide banding patterns for 1α, 2α, α2(V), and α1(V) (lanes 2–5) all differed from each other, suggesting that these chains are the products of different collagen genes. The CNBr peptides derived from HMW also did not appear related to those of any other chain (lane 1). In additional experiments, we have examined the peptides derived from chicken α1(I), α2(I), and α1(III) chains and have been unable to recognize any similarities to peptides derived from 1α, 2α, α2(V), α1(V), and HMW (C. A. Reese and R. Mayne, unpublished observations).

The peptides obtained after limited digestion of the samples with *S. aureus* V8 protease were also analyzed by polyacrylamide gel electrophoresis (Figure 6). The banding patterns for the α1(II) and 3α chains again showed marked similarities to each other (lanes 1 and 2), with all of the peptides derived from 3α migrating more slowly than those derived from the α1(II) chain. The peptides derived from 2α, 1α, α1(V), and α2(V) all gave different banding patterns (lanes 3–6). However, some similarities were noted when the banding patterns for 1α and α1(V) chains were compared (lanes 4 and 5), although the overall patterns appeared different.

Several additional experiments were performed to demonstrate that highly purified preparations of chick chondrocytes selected as "floaters" synthesize 1α, 2α, and 3α. Polyacrylamide gel electrophoresis and subsequent fluorographic analysis of the radioactively labeled collagen present in the precipitates obtained at 0.9 M NaCl–0.5 M HOAc and 1.2 M NaCl–0.5 M HOAc are shown in Figure 7. The predominant collagen present in the 0.9 M NaCl precipitate was identified as α1(II) (lane 1) whereas three prominent bands were observed in the 1.2 M NaCl precipitate which corresponded in migration positions to 1α, 2α, and 3α (lanes 2 and 3). Overexposure of the gel during fluorography also resulted in the appearance of bands corresponding in location to HMW and LMW, and these bands were no longer present with reduction (data not presented). Further characterization of the 1.2 M NaCl precipitate was carried out by CM-cellulose chromatography (Figure 8). Three prominent peaks of radioactivity were observed, which eluted slightly after the carrier

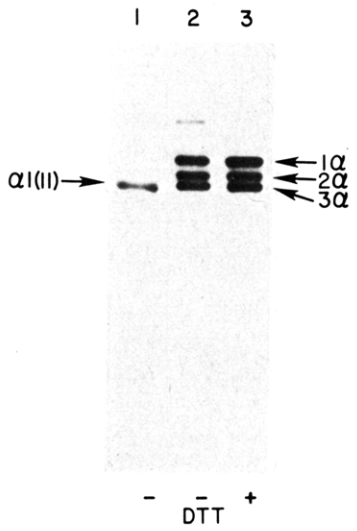

FIGURE 7: Fluorograph prepared after $NaDodSO_4$–polyacrylamide gel electrophoresis (5–7.5% gradient gel) of the collagen synthesized by cultured chick chondrocytes and separated by differential salt precipitation in the presence of carrier collagens. Lanes were (1) the precipitate at 0.9 M NaCl–0.5 M HOAc and (2 and 3) the precipitate obtained at 1.2 M NaCl–0.5 M HOAc without and with reduction (50 mM dithiothreitol, 100 °C, 1 min).

3α, 1α, and 2α chains present in the 1.2 M NaCl precipitate. Each of the three peaks was desalted, lyophilized, and analyzed by polyacrylamide gel electrophoresis followed by fluorography. The radioactivity present in each peak was shown to correspond in migration to the positions of authentic 3α, 1α, and 2α chains.

Discussion

Our results show that, in addition to type II collagen, small amounts of several other collagenous molecules are present in chicken hyaline cartilage and are solubilized after limited pepsin digestion. Three chains have been isolated which appear identical with the 1α, 2α, 3α chains described by Burgeson & Hollister (1979), with the 3α chains appearing from CNBr and V8 protease peptide maps to be closely related to the α1(II) chain. The 1α and 2α chains from peptide mapping experiments appear unique, although these chains are clearly closely related to the α1(V) and α2(V) chains both in amino acid composition and in migration position on polyacrylamide gel electrophoresis. Recently, it was proposed that both CNBr and protease peptide mapping experiments must be performed in order to identify new collagen chains (Bornstein & Sage, 1980). By these criteria, we have established that 1α and 2α are different chains from the α1(V) and α2(V) chains of type V collagen. However, some of our peptide maps do show similarities [e.g., the V8 protease peptides of 1α and α1(V); Figure 6], and we consider that the final proof that 1α and 2α are different chains from α1(V) and α2(V) will only come from a detailed analysis of the CNBr peptides derived from each chain, together with some limited amino acid sequencing.

In addition to 1α, 2α, and 3α, we have also isolated two new collagenous molecules from chicken hyaline cartilage which we call HMW and LMW. Both of these molecules are very similar in amino acid composition to 1α, 2α, α1(V), and α2(V) but differ in that they contain disulfide bridges. It is possible that LMW is derived from HMW by further pepsin cleavage. However, we have been unable to generate LMW from HMW by further incubation of the 2.0 M precipitate with pepsin (C. A. Reese and R. Mayne, unpublished observations). HMW after reduction gave rise to three components which we call C-1, C-2, and C-3, which possess apparent M_r of 87 500, 51 000, and 36 400, respectively, as determined by agarose gel filtration. After reduction, the major component is C-2, and our molecule therefore appears much larger than the similar molecule isolated by Shimokomaki et al. (1980) and Ayad et al. (1981) from mammalian cartilages, in which a molecule consisting of three identical chains of M_r 33 000 was proposed. This apparent difference may, however, be a reflection of the location of additional pepsin-sensitive sites within the mammalian molecule, which are not cleaved in the chicken. The M_r of LMW was 30 000, as determined by agarose gel filtration, and after reduction, LMW gave rise to two components of apparent M_r 20 000 and 10 000.

All experiments so far performed with chicken cartilage have used limited pepsin digestion in order to solubilize the collagens, and it is therefore possible that only pepsin-resistant domains of much larger molecules are being extracted. Recently, it has been shown that the final processed forms of the α1(V) and α2(V) chains synthesized by the chicken crop or human placenta in organ culture are considerably larger than the chains obtained after pepsin digestion (Kumamoto & Fessler, 1980; Foidart et al., 1981). We cannot therefore exclude the possibility that HMW or LMW and the 1α or 2α chains are present in the same molecule or molecules but are separated by pepsin-sensitive sites. A precedent for this possibility already exists for type IV collagen, where 7S collagen is a disulfide-bonded domain which is separated from the remainder of the molecule by a pepsin-sensitive region (Kühn et al., 1981).

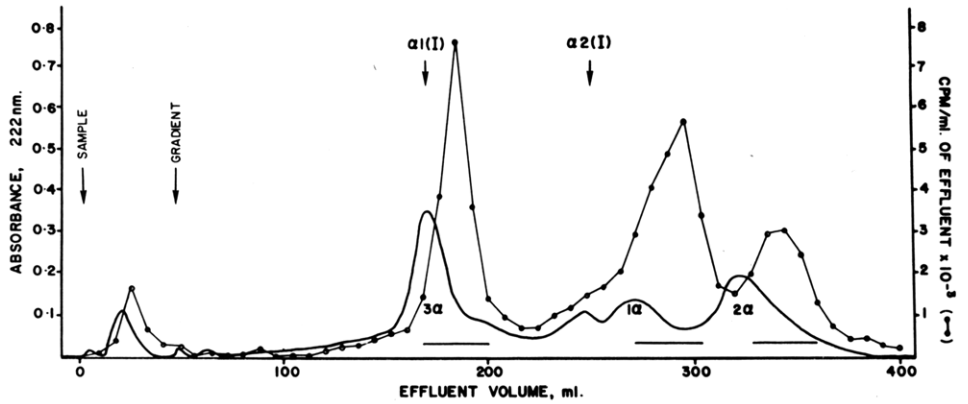

FIGURE 8: CM-cellulose elution pattern of radioactively labeled collagen recovered from chick chondrocytes after pepsin digestion as the precipitate at 1.2 M NaCl–0.5 M HOAc (see Figure 7, lane 2). The carrier was prepared from adult chicken cartilage after differential salt precipitation as the 1.2 M NaCl precipitate and contained 3α, 1α, and 2α. Bars indicate fractions which were pooled for further analysis by fluorography after polyacrylamide gel electrophoresis.

In the present series of experiments, no attempt has been made to determine the yields of the various collagenous components. However, the precipitate obtained at 1.2 M NaCl–0.5 M HOAc represented by weight less than 1% of the total solubilized collagen whereas the precipitate obtained at 2.0 M NaCl–0.5 M HOAc represented 5% of the total collagen. In view of these low proportions, experiments were performed to demonstrate that 1α, 2α, and 3α are synthesized by chick chondrocytes grown in tissue culture. Highly purified populations of chondrocytes were derived from chick embryonic sterna as floaters, and the experiments described in Figures 7 and 8 show that 1α, 2α, and 3α are synthesized by the chondrocytes themselves and not by another cell type.

At present the α1(V), α2(V), and α3(V) chains are all considered to represent the chains of type V collagen, although the molecular organization of these chains still remains to be established [reviewed by Bornstein & Sage (1980)]. We would like to suggest that 1α, 2α, and HMW should also be included in the type V collagen family, the cartilage chains clearly being closely related to the type V collagen chains isolated from other tissues both in amino acid composition and in being soluble in the native state at high salt concentrations both in neutral and acidic conditions. We have failed to isolate 1α, 2α, and HMW from pepsin digests of chicken gizzards or kidneys (R. Mayne, unpublished observations), and cartilage may therefore possess a matrix not only constructed from type II collagen but also requiring 1α, 2α, and HMW for the stabilization of the matrix. If so, there may be several collagenous molecules, the genes for which are perhaps uniquely expressed in cartilage cells. It will be important to determine if, during the switching from the synthesis of type II collagen to the synthesis of type I collagen which occurs in chick chondrocyte cultures (Mayne et al., 1975), a similar switch will occur from the synthesis of 1α, 2α, and HMW to the synthesis of α1(V) and α2(V).

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