CHARACTERIZATION OF NOTOCHORD COLLAGEN AS A CARTILAGE-TYPE COLLAGEN*

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SUMMARY: Sturgeon notochord and cartilage collagens have been characterized with respect to chromatographic properties, amino acid composition, carbohydrate content, and cyanogen bromide cleavage products of the component α chains. The data show that the collagen of both tissues is comprised of a single type of α chain and that the notochord and cartilage chains are identical. Further, the sturgeon chains bear a striking resemblance to previously characterized α1(II) chains from avian and mammalian hyaline cartilages. These observations strongly suggest that the data may be extrapolated to higher organisms and indicate that during development, a cartilage-type collagen is synthesized by notochord cells prior to the appearance of tissues classically identified as cartilage on the basis of morphology.

Recent investigations have demonstrated that the collagen fibers in several hyaline cartilages are comprised of a unique type of collagen molecule containing three α1(II) chains and therefore exhibiting the chain composition $[\alpha 1(II)]_3$ (1-7). The latter chains differ in amino acid composition and primary structure from α1(I), α2, and α1(III) chains which exist as molecules with chain compositions, $[\alpha 1(I)]_2\alpha 2$ (8) and $[\alpha 1(III)]_3$ (9), in other connective tissues.

This information with respect to collagen polymorphism as well as further observations suggesting that collagen could be involved in embryonic interactions (10-12) has stimulated a renewed interest in the possibility that collagen may

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serve as an inducer in certain types of embryonic differentiation (13). The neural tube and notochord, for instance, appear to promote medial migration and chondrogenesis on the part of somitic mesenchyme cells (14). It is of interest, then, that both of these tissues in organ culture produce a collagen containing only a single type of α chain (15,16). Moreover, in the case of notochord, the labeled collagen chains produced in organ culture exhibited chromatographic properties compatible with their tentative identification as α1(II) chains (16).

In the present communications we present direct chemical evidence that sturgeon notochord and cartilage collagens are chemically identical indicating that the corresponding homologous collagens in higher vertebrate species may also be identical. In addition, the data indicate that α1(II) chains derived from sturgeon tissues bear a striking resemblance in amino acid composition and cyanogen bromide (CNBr) cleavage products to previously studied avian (17), and mammalian (7) α1(II) chains.

MATERIALS AND METHODS. Sturgeon (Scaphirhynchus platorhynchus) notochord and cartilage collagen were solubilized from the respective tissues (previously extracted with 4 M guanidinium chloride) by limited digestion with papain at low temperature (4). Purification of the collagen solubilized during this procedure was achieved by successive precipitation with ammonium sulfate, by dialysis against 0.02 M dibasic sodium phosphate, by the addition of NaCl to neutral salt solutions of the collagen, and finally by the addition of NaCl to the collagen dissolved in 0.5 M acetic acid. The final precipitate was dissolved in 0.5 M acetic acid, centrifuged at 100,000 x g, and lyophilized. It was estimated on the basis of the amount of collagen recovered after the final precipitation that the papain digestion procedure had solubilized approximately 50% of the collagen in each type of tissue.

The collagen from both tissues was dissolved in starting buffer, denatured, and chromatographed on carboxymethyl cellulose as previously described (3). The chains eluted from carboxymethyl cellulose were subsequently cleaved with cyanogen bromide in 70% formic acid (17), and the resulting peptide fragments

were resolved by chromatography on carboxymethyl cellulose (18). Molecular weights of the α chains and cyanogen bromide cleavage products were estimated on a calibrated agarose molecular sieve column (18), and amino acid analyses were performed on an automatic amino acid analyzer employing a single-column procedure (18).

Analyses of the amount and nature of the carbohydrate prosthetic groups associated with the purified sturgeon α chains were performed as described previously (19).

RESULTS AND DISCUSSION. A carboxymethyl cellulose chromatogram illustrating the elution pattern of denatured sturgeon notochord or cartilage collagen is given in Figure 1. The collagen from both tissues was readily soluble in the

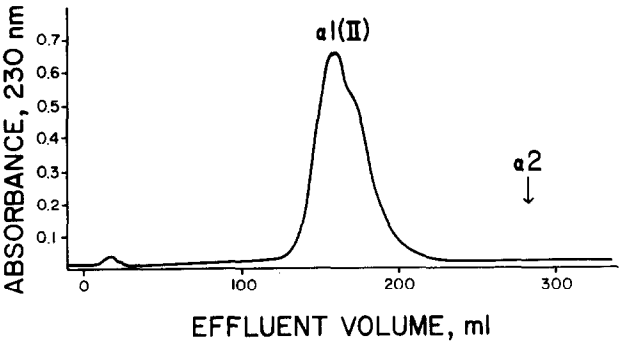

Figure 1: The carboxymethyl cellulose elution pattern of 75 mg of denatured sturgeon notochord or cartilage collagen. Chromatography was performed at 42° C on a 1.8 x 10 cm column. Elution was achieved at a flow rate of 100 ml/hr. in 0.04 M (Na^+) sodium acetate, pH 4.8, containing 1.0 M urea by employing a linear gradient from 0.0 to 0.12 M NaCl over a total volume of 400 ml.

starting buffer for chromatography, and essentially quantitative recovery of the material applied to the column was achieved. The chromatogram is characterized by a single somewhat asymmetric peak in the α1 chain position and the absence of material chromatographing in the region where α2 chains are eluted. These features are characteristic of a cartilage-type collagen from avian (2,3) and mammalian (4) sources. The protein eluted from carboxymethyl cellulose was

further characterized by rechromatography on a calibrated agarose molecular sieve column. The results indicated that all of the protein eluted from carboxymethyl cellulose (for both notochord and cartilage collagen) was in the form of α chains with a molecular weight of 95,000 daltons.

Amino acid analyses of the notochord and cartilage collagen α chains prepared as described above are presented in Table 1. These data indicate that the collagen α chains from both tissues have the same amino acid composition and therefore represent products of the same genetic locus. Moreover, these chains exhibit many of the compositional features previously observed for

Table I

Amino Acid Composition of Notochord and Cartilage Collagen α Chains and Selected Cyanogen Bromide Peptides.

	Residues/1,000 Residues (α Chains)		Residues/Peptide (Peptides)	
Amino Acid	Cartilage	Notochord	(6,12)[b]	11
3-Hydroxyproline	2	2	0	0
4-Hydroxyproline	88	86	12	25
Aspartic Acid	46	46	4	10
Threonine	20	20	2	5
Serine	38	38	4	8
Glutamic Acid	89	89	11	23
Proline	118	121	13	31
Glycine	338	336	39	93
Alanine	106	105	12	31
Valine	14	14	0	3
Methionine[a]	4	4	1	1
Isoleucine	8	8	1	3
Leucine	27	28	3	7
Tyrosine	1	1	1	0
Phenylalanine	12	13	2	4
Hydroxylysine	19	21	3	6
Histidine	3	3	1	2
Lysine	16	14	3	2
Arginine	51	51	5	16
Total	1,000	1,000	117	270

a. Indicates homoserine for cyanogen bromide peptides.

b. Designated peptide (6,12) since it has a size and amino acid composition indicating it accounts for the sequences represented by peptides 6 plus 12 in avian (17) and mammalian (7) α1(II) chains.

α1(II) chains in higher vertebrate species (2-4,7). These features include relatively high contents of glutamic acid, leucine, and hydroxylysine, and a relatively low content of alanine. As also noted for α1(II) chains in other species (2-4), the relatively high content of hydroxylysine in the sturgeon chains is associated with relatively large amounts of carbohydrate. Characterization of the sturgeon notochord and cartilage α chains with respect to carbohydrate content revealed the presence of only D-glucose and D-galactose each of which were present at the level of approximately 14 residues per chain. These results are consistent with the presence of approximately 14 residues of glucosylgalactosylhydroxylysine in these chains.

In order to further characterize the α chains from sturgeon tissues and examine their relationship to α1(II) chains of other species, the sturgeon chains were cleaved with cyanogen bromide, and the resulting peptides were chromatographed on carboxymethyl cellulose. The results of these studies confirmed the conclusions cited above with respect to the identity of the cartilage and notochord chains, since the elution patterns for peptides derived from each chain were identical. A representative chromatogram is shown in Figure 2. This elution pattern differs from those previously observed in similar studies on avian (17) and mammalian (7) α1(II) chains, since the sturgeon chains contain fewer methionyl residues. Nevertheless, two of the sturgeon peptides, designated (6,12) and 11 (Figure 2) could be readily identified on the basis of chromatographic position, size, and amino acid composition (Table 1) as possible homologs of peptides with the same designation from avian (17) and mammalian (7) α1(II) chains. The carboxymethyl cellulose eluant between 600 and 800 ml (Figure 2) contains two additional peptides which account for virtually all of the remaining molecular weight and amino acids of the sturgeon α chains. The latter peptides, however, have no obvious counterparts in the cyanogen bromide cleavage products of α1(II) chains from other species.

The present data demonstrate, at least for the sturgeon, that notochord and cartilage collagen are chemically identical. Moreover, the similarity of

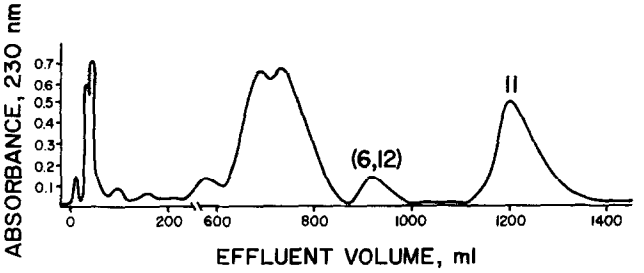

Figure 2: The carboxymethyl cellulose elution pattern of 200 mg of cyanogen bromide peptides derived from sturgeon notochord or cartilage collagen α chains. Chromatography was performed at 42° C on a 2.5 x 10 cm column. Elution was achieved at a flow rate of 200 ml/hr. in 0.02 M (Na^+) sodium citrate, pH 3.6, by employing a linear gradient from 0.01 to 0.16 M NaCl over a total volume of 2,000 ml.

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sturgeon notochord and cartilage α chains to α1(II) chains of higher vertebrates with respect to amino acid composition and cyanogen bromide cleavage products strongly suggests that this data may be extrapolated to other species as well. In addition, the data indicate that during development of the vertebrate embryo, a cartilage type collagen is synthesized by notochord cells prior to the appearance of tissues classically defined as cartilage on the basis of morphology. Thus, notochord cells may represent the first embryonic cells programmed to elaborate and secrete the type of collagen found only in hyaline cartilages of most adult vertebrates. These data, of course, do not establish a direct cause and effect relationship between the presence of a cartilage-type collagen in notochord and subsequent chondrogenesis on the part of adjacent somite cells. They do, however, suggest that the inductive interaction between these tissues could be highly dependent on the collagenous component of the notochord and tissue culture experiments designed to test this hypothesis should prove feasible.

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