# HETEROGENEITY IN THE CYANOGEN BROMIDE PEPTIDES FROM STRIATED MUSCLE AND HEART VALVE COLLAGEN

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SUMMARY

Cyanogen bromide cleavage of insoluble striated muscle and heart valve collagen has revealed the presence of heterogeneous peptides in these tissues. A peptide homologous in molecular weight to α1CB3 of soluble collagen was isolated from insoluble muscle, heart valve and cartilage collagen. The amino acid composition of these peptides indicated however, that they were not identical. A peptide homologous to a α1CB4 plus α1CB5 methionine substituted peptide was also found in insoluble muscle collagen and a peptide very similar to peptide nine of cartilage was found in heart valve collagen. These results suggest that two or more genetically distincttypes of α1-chain are present in these collagens.

In the past few years investigators have presented evidence showing the existence of genetically distinct types of α-chains in a number of collagens (1,2,3,4). Cartilage collagen, for example, appears to be composed of a mixture of two different collagen molecules, one having the chain structure normally found in bone and skin (α1)2α2 and the other comprised of three identical chains [α1(II)]3 (5). An analogous situation has been reported for human skin collagen with the chains in this instance designated as α1 Type III (2).

In this communication we report heterogeneities in the cyanogen bromide (CNBr) peptides of collagen from insoluble striated muscle and heart valve, which suggest the existence of additional chain types.

METHODS AND MATERIALS. Cartilage was obtained from the processus spinosus (spinous processes) of the 6th and 7th cervical and 1st through 5th thoracic vertebra of veal calves. Atrioventricular valves were obtained from the hearts of animals of similar age. Intramuscular connective tissue was isolated from calf longissimus muscle by methods previously described (6) with the exception that the muscle samples were lyophilized before blending. The collagen samples were extracted for two days with two portions of 1 M NaCl, 0.05 M tris, and subsequently with two portions of 0.5 M acetic acid for two consecutive days. The soluble muscle collagen was purified by salt precipitation and dialysis according to previously reported methods (7). This extraction procedure for heart valve and cartilage failed to solubilize any significant amounts of collagen. Following the last extraction procedure the insoluble collagens from these four sources were washed thoroughly with water, lyophilized, and ground in a Wiley Mill.

The technique reported by Miller (2) was utilized for CNBr cleavage with the exception that the incubation temperature was raised to 40°C. Purified acetic acid soluble muscle collagen was digested without prior separation of the chains. The peptide containing material was either eluted with 0.1 M acetic acid through a 6 x 40 cm column of Bio-Gel P-2 (100-200 mesh) or lyopholized twice to assure removal of CNBr and salts.

The CNBr peptides were chromatographed on 1.8 x 10 cm column of CM-cellulose (5). Purification and molecular weight determination of the isolated peptides was accomplished by rechromatography on phosphocellulose or chromatography on a 1.5 x 110 cm calibrated agarose column (5). Amino acid composition of the peptides was determined by methods described previously (9). The nomenclature applied to the CNBr peptides follows that outlined by Miller et al (8).

RESULTS. Approximately 80% of the insoluble muscle collagen was recovered as dry weight material after the CNBr reaction. In the case of soluble muscle collagen, essentially complete recovery was obtained within two hours after

initiating the reaction with CNBr. Only 50% of the material derived from the insoluble heart valve and cartilage collagen could be recovered from the P-2 eluant.

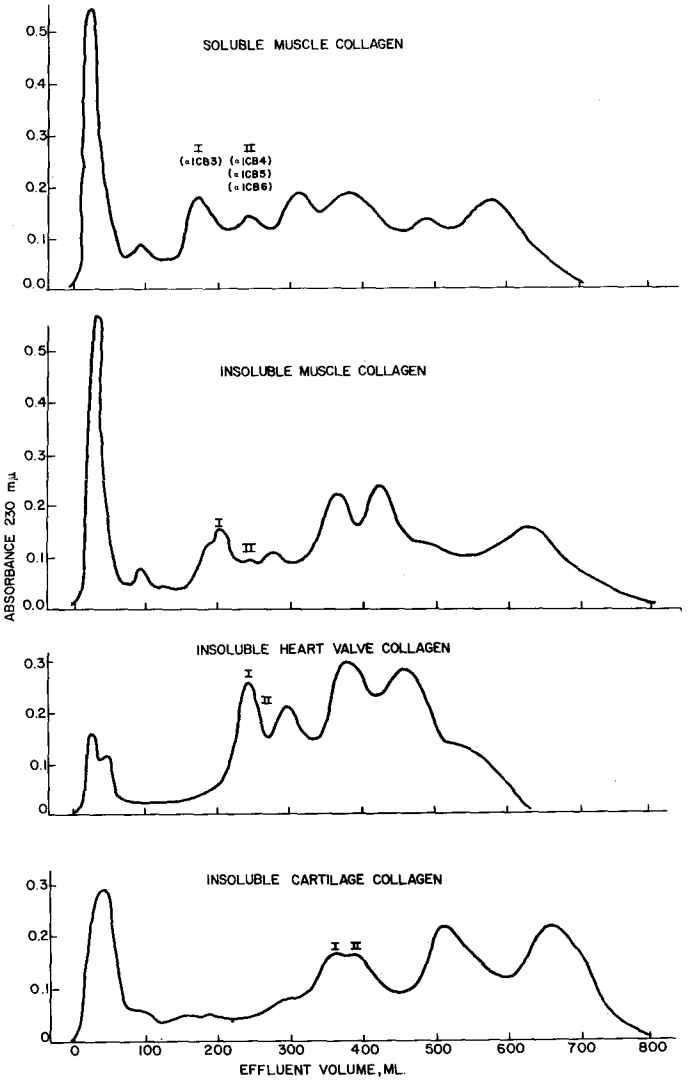


Fig. 1. CM-Cellulose chromatograms of the CNBr peptides from soluble muscle, insoluble muscle, heart valve and cartilage collagen.

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Representative CM-cellulose chromatograms of the CNBr peptides obtained from the four collagen sources are shown in Figure 1. The peptides from

soluble muscle collagen were present in quantities indicative of a 2:1 molar ratio of α1 and α2 chains. The α1CB6 and several other peptides from insoluble muscle collagen were not present in stoichiometric quantities, and several new peptides were observed. Although not always present, a peptide eluting slightly later than α1CB6 in the insoluble muscle collagen chromatograms, had a molecular weight slightly greater than α1CB6 and contained an additional 17 amino acids. This peptide contained two residues of tyrosine, suggesting its possible participation in an end to tail type cross-link. The peptide elution patterns observed for cartilage and heart valve were markedly different and less complex than those commonly seen for soluble collagen. No peptide corresponding to α1CB6 was found in either of these insoluble collagen sources.

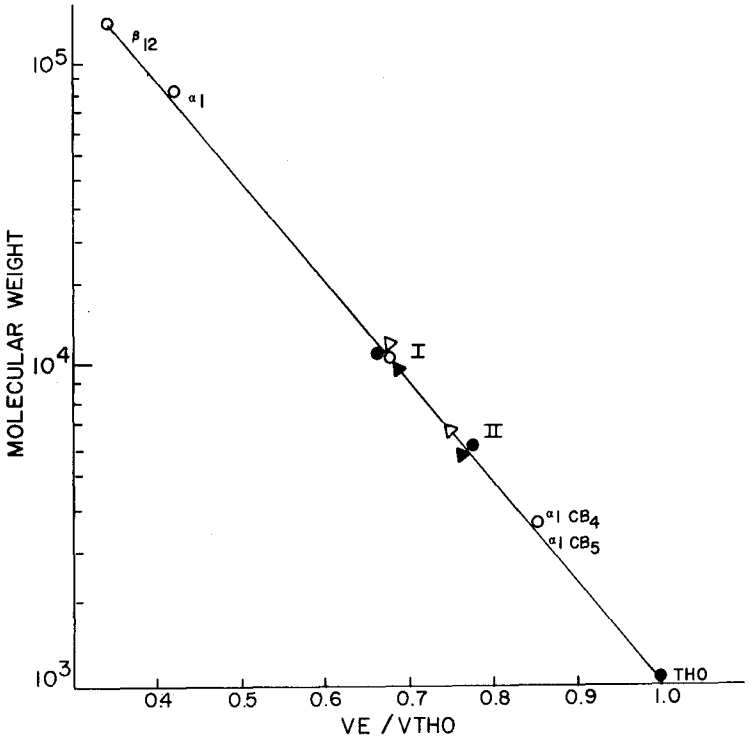


Fig. 2. Plot of log molecular weight vs. elution volume, Ve/VTHO, of Peak I and II peptides.
O = soluble muscle, Δ = insoluble muscle, ● = heart valve, ▲ = cartilage.

The peptides represented by peak I (Figure 1.) for all the samples were isolated and rechromatographed or purified by molecular sieve chromatography. The peak I peptides from all the collagens had almost identical molecular weights as determined by chromatography on an equilibrated agarose column (Figure 2) indicating a close homology to the α1CB3 peptide of soluble collagen. However, the heterogeneity of these peptides is evident from the amino acid composition (Table I). The peak I peptide from insoluble muscle collagen had a higher hydroxyproline and lysine content and lower quantities of alanine and valine than that from soluble muscle collagen. An isoleucine-leucine interchange was also apparent. The heart valve and cartilage peak I peptides contained higher amounts of threonine and serine, reduced quantities of alanine and the lysine residues were more highly hydroxylated than the

TABLE I

Amino Acid Composition of Peak I Peptides[a] From Soluble Muscle, Insoluble Muscle, Heart Valve And Cartilage Collagen

| | Soluble Muscle | Insoluble Muscle | Heart Valve | Cartilage |
|---|---|---|---|---|
| Aspartic Acid | 6(6.2) | 6 | 6(6.2) | 5(5.3) |
| Hydroxyproline | 15 | 17 | 18 | 17 |
| Threonine | 1(0.9) | 1(1.2) | 2 | 3 |
| Serine | 3(3.3) | 3(3.2) | 6(6.2) | 4(4.2) |
| Glutamic | 14 | 14 | 11 | 13 |
| Proline | 15 | 15 | 15 | 15 |
| Glycine | 48 | 48 | 48 | 48 |
| Alanine | 20 | 17 | 15 | 15 |
| Valine | 4(3.6) | 3(3.2) | 3(2.6) | 3(2.6) |
| Isoleucine | (0.3) | 1(0.8) | 2(2.2) | 1(1.2) |
| Leucine | 4(3.8) | 3(3.1) | 3(2.5) | 5(5.3) |
| Phenylalanine | 3(3.1) | 3(2.7) | 2(1.8) | 3(2.6) |
| Hydroxylysine | 0.5 | 0.7 | 2 | 2.5 |
| Lysine | 4.9 | 6.3 | 4.9 | 3.8 |
| Histadine | 0 | 0 | 0 | 0 |
| Arginine | 6(6.4) | 6(6.2) | 6(6.2) | 6(6.3) |
| Homoserine[b] | 1(0.9) | 1(0.9) | 1 | 1(0.9) |
| Total | 145 | 145 | 145 | 145 |

[a] Actual values are listed where less than 10 residues are found. A zero indicates less than 0.2 residues.

[b] Includes homoserine lactone.

muscle collagen peptides. The number and magnitude of the amino acid differences between the heart valve and cartilage peak I peptides also clearly indicate tissue specific variation.

Peak II from soluble muscle collagen contained the peptides α1CB4, α1CB5 and α1CB6. These peptides were also present in varying amounts in the peak eluting immediately after peak II from insoluble muscle collagen (Figure 1). The additional peak found in chromatograms of insoluble muscle collagen (Peak II) contained a peptide which was not, however, found in soluble muscle collagen. This peptide appeared to have the same relationship to α1CB4 and α1CB5 as the α1(III)CB (4,5) methionine substituted peptide isolated from human insoluble skin collagen (2). The insertion of a residue

TABLE II

Amino Acid Composition of Peak II Peptides[a] From Soluble Muscle,[b] Insoluble Muscle, Heart Valve And Cartilage Collagen

| | Soluble Muscle | Insoluble Muscle | Heart Valve | Cartilage |
|---|---|---|---|---|
| Aspartic Acid | 5 | 5(4.7) | 4 | 4(3.8) |
| Hydroxyproline | 10 | 8(7.8) | 8(8.3) | 8(8.2) |
| Threonine | 2(1.8 | 2(1.6) | 1(1.2) | 1(1.3) |
| Serine | 2(2.1) | 3(3.1) | 3(3.1) | 2(1.8) |
| Glutamic | 6(5.7) | 6 | 5(4.9) | 5(4.7) |
| Proline | 7(7.1) | 9 | 7(7.2) | 7(7.2) |
| Glycine | 28 | 28 | 23 | 23 |
| Alanine | 8(7.4) | 7 | 6(6.2) | 5(5.2) |
| Valine | 0 | 1(0.9) | 1(1.2) | 1(0.8) |
| Isoleucine | 0 | 1(1.2) | 1(0.8) | (0.2) |
| Leucine | 3 | 2(2.3) | 2(2.3) | 3(2.7) |
| Phenylalanine | 1(0.7) | 1(0.9) | 1(1.1) | 1(0.8) |
| Hydroxylysine | 0.7 | 1.2 | 1.2 | 0.8 |
| Lysine | 3.5 | 3.7 | 3.2 | 2.8 |
| Histidine | 1(0.8) | 1(0.7) | 1(0.9) | 1(0.7) |
| Arginine | 4 | 4 | 4 | 6 |
| Homoserine[c] | 2(1.9) | 1(0.9) | 1(0.9) | 1 |
| Total | 84 | 84 | 72 | 72 |

[a] Actual values are listed where less than 10 residues are found. A zero indicates less than 0.2 residues.

[b] Represents analysis of α1CB4 plus α1CB5.

[c] Includes homoserine lactone.

each of serine and valine and the substitution of an isoleucine for a leucine in the new peptide clearly differentiate it from the sequence from soluble muscle collagen represented by $\alpha$1CB4 plus $\alpha$1CB5 (Table II).

A unique peptide, eluting in a similar position in the CM-cellulose chromatogram, was also found in heart valve collagen. This peptide had a molecular weight of 6500 as determined by agarose chromatography (Figure 2.) and contained 12 residues less than the peptide isolated from insoluble muscle collagen (Table II). A similar peptide eluting slightly later in the chromatogram was also present in cartilage collagen. Based on molecular weight and amino acid composition, these peptides appear to be closely homologous to the peptide 9 described by Miller (5) for chick sternal cartilage. Substitution of residues of serine, alanine and isoleucine and deletions of one residue of leucine and two of arginine differentiate the heart valve peptide from cartilage.

DISCUSSION. Analogous to the situation in insoluble human cartilage and skin collagen, it appears likely that the new peptides from insoluble bovine muscle, cartilage and heart valve collagen are derived from genetically distinct $\alpha$-chains. Based on the ratio of either $\alpha$1CB4 or $\alpha$1CB5 and the new peptide from insoluble muscle collagen, approximately 60% of this collagen would be composed of the new type molecules. The analogous CM-cellulose elution patterns and the obvious similarity of the peak II peptides suggests a great degree of structural homology between the cartilage and heart valve collagen. Since spinous process cartilage is intimately involved in osteogenesis, this homology may be of significance in explaining the degenerative fibro-calcific damage observed in heart valves.

In any case, it now appears that the new type $\alpha$-chains may be more widely distributed throughout the collagenous tissues than previously thought. The existence of these genetically distinct series of collagens suggests that they may be specifically synthesized, possibly in varying amounts, for adaptation to tissue function. Failure to regulate differential collagen

synthesis could play a fundamental role in the pathogenesis of connective tissue disorders.

## REFERENCES


1. Miller, E.J. and Matukas, V. J., Proc. Natl. Acad. Sci, U. S., 64, 1264 (1969).
2. Miller, E.J., Epstein, E.H. and Piez, K. A., Biochem. Biophys. Res. Comm. 42, 1024 (1971).
3. Telstad, R.L., Kang, A.H., Igarashi, S. and Gross, J., Biochemistry 9, 4993 (1970).
4. Strawich, E. and Nimni, M.E., Biochemistry, 10, 3905 (1971).
5. Miller, E.J., Biochemistry, 10, 3030 (1971).
6. McClain, P.E., Nature, 221, 181 (1969).
7. McClain, P.E. and Wiley, E.R., J. Biol. Chem., 247, 698 (1972).
8. Miller, E.J., Lane, J.M. and Piez, K.A., Biochemistry, 8, 30 (1969).
9. McClain, P.E., Creed, G.J., Wiley, E.R. and Gerrits, R.J., Biochem. Biophys. Acta, 221, 349 (1970).