Cross-Linking Characteristics of Cardiac Muscle Collagen (37484)

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Jackson (1) has pointed out the need for studies of connective tissues other than the conventional skin and tendon in order to establish the patterns of *in vivo* collagen fibrogenesis. Efforts in this laboratory have been directed primarily toward the study of collagen from the intramuscular connective tissues isolated from striated muscle (2). Collagen from this source has proven to be a unique type whose physical-chemical and cross-linking characteristics vary not only from species to species (3) but also differ markedly from muscle to muscle (4).

We have subsequently extended our studies to collagen from interstitial connective tissues of cardiac muscle. Initial attempts to isolate cardiac collagen utilizing the method employed for striated muscle (5) yielded only traces of cardiac connective tissue. We wish to describe here an improved method for the isolation of both striated and cardiac muscle connective tissue and to report the results of studies on the cross-linking characteristics of cardiac collagen.

Materials and Methods. Fresh rat and bovine cardiac muscle was dissected free of adhering fat, valves, blood vessels, tendons and visible connective tissue, and frozen on dry ice. The isolation method previously described (5) was modified in that samples were lyophilized prior to fragmentation. All subsequent operations were carried out in a cold room kept at 0 to 4°. The dried samples were blended with dry ice in a high speed commercial blender for 1–2 min to reduce particle size. The samples were then further fragmented with dry ice in a 1 quart stainless steel blender for 2–3 min at approximately 14,000 rpm. Due to the relative ease of fragmentation of the dried muscle tissues, only short blending periods were required. The finely powdered muscle mass was then separated from the connective tissue by passing the blended samples through a size 10 mesh sieve. The white fibrous mass of connective tissue obtained from the top of the sieve was almost completely free of adhering muscle tissue. Connective tissues from bovine striated muscle, rat skin and rat tail tendon were isolated in a similar manner.

Neutral salt- and acid-soluble tropocollagen were prepared by extracting the isolated connective tissues with 0.45 *M* NaCl at 2° for two periods of 24 hr each and then with 0.5 *M* acetic acid for similar periods (4). Aliquots were removed from the two soluble fractions and the hydroxyproline content was determined by the method of Woessner (6). The salt- and acid-soluble fractions were purified by salt precipitation and dialysis against 0.02 *M* disodium phosphate as previously outlined (3). Guanidine-soluble or lyotropic collagen was isolated and purified from the residue obtained from the above extracts by the methods outlined by Veis *et al.* (7) and Miller *et al.* (8). The insoluble residue was defatted and dried by washing with ethanol and ether, and further purified according to the method of Jackson and Cleary (9) to a final hydroxyproline content of 13.5%.

The soluble collagen fractions were subjected to acrylamide gel electrophoresis (10) or carboxymethyl cellulose chromatography (8). The amino acid composition of collagen samples was determined as previously outlined (3).

Results and Discussion. Utilizing this isolation method, the yields of muscle connective tissue were increased to approximately 6% of the total muscle mass as compared to 2–3% by the previous method (5). The quantity of soluble tropocollagen obtained from

TABLE I. Amino Acid Composition[a] of Skin and Cardiac Collagens.

Amino acid	Rat		Bovine	
	Skin	Cardiac	Skin	Cardiac
Aspartic acid	48	57	45	46
4-Hydroxyproline	91	103	94	101
Threonine	18	21	18	16
Serine	39	48	36	35
Glutamic acid	71	83	72	70
Proline	116	95	138	128
Glycine	337	328	320	339
Alanine	99	88	112	102
Valine	24	25	20	23
Methionine	6	8	4	8
Isoleucine	14	16	11	16
Leucine	26	29	25	26
Tyrosine	4	5	3	4
Phenylalanine	12	13	13	13
Hydroxylysine	6	12	7	11
Lysine	29	23	27	23
Histidine	6	5	5	5
Arginine	48	37	50	40

[a] Results represent mean values from three samples expressed as residues per 1000 residues.

striated muscle connective tissue was identical to that previously reported (11), indicating that organizational structure of the muscle collagen had not been altered in the modified isolation process. Cardiac muscle connective tissues were obtained in quantities of 6% of the dry muscle mass and were composed of approximately 80% collagen as determined by hydroxyproline analysis.

The amino acid composition of intact rat and bovine cardiac collagen, purified by the method of Jackson and Cleary (9), is shown in Table I, with rat and calf skin amino acid composition given for comparison. Although the amino acid composition of rat and bovine cardiac collagen was in general typical of that reported for other mammalian collagens, some marked variations were evident. Rat cardiac muscle collagen was higher in aspartic and glutamic acid and in serine than rat skin collagen. Both rat and bovine cardiac collagen had higher quantities of isoleucine and methionine and lower amounts of alanine and arginine than the corresponding values from rat and bovine skin. Although the total amino acid and lysine plus hydroxylysine content was approximately the same, there was a greater degree of hydroxylation in the cardiac collagens as reflected by the higher hydroxyproline and hydroxylysine content.

Table II shows the yields of salt- and acid-soluble tropocollagen obtained from skin, tendon, striated and cardiac muscle collagens.

TABLE II. Solubility[a] of Rat and Bovine Collagens.

Collagen source	Tropocollagen	
	Salt-soluble	Acid-soluble
Rat tail tendon	4.3	20.0
Rat skin	6.2	17.1
Rat cardiac	0.9	1.9
Bovine striated muscle	6.0	10.0
Bovine cardiac	<0.1	<0.1

[a] Percentage of the total collagen.

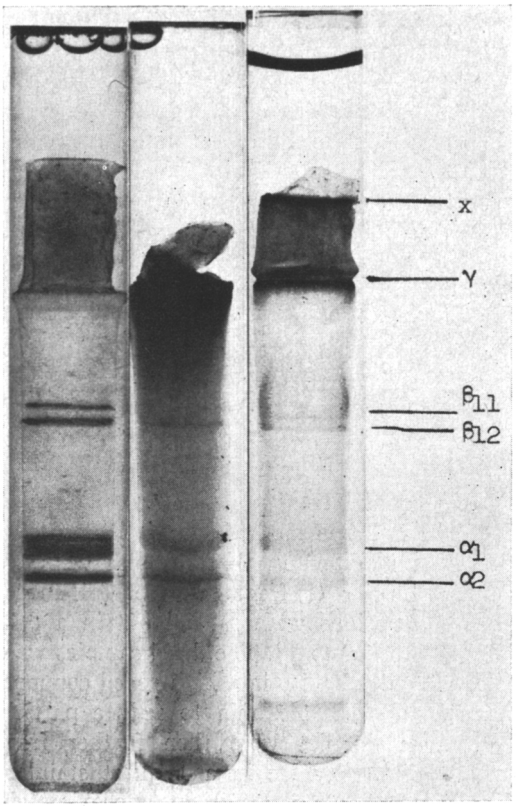

FIG. 1. Disc gels from acid-soluble tropocollagen. From left to right, rat skin, bovine cardiac, rat cardiac. $\alpha 1$ and $\alpha 2$ = monomers, β_{12} and β_{11} = dimers, γ = trimer, x = higher molecular weight aggregates.

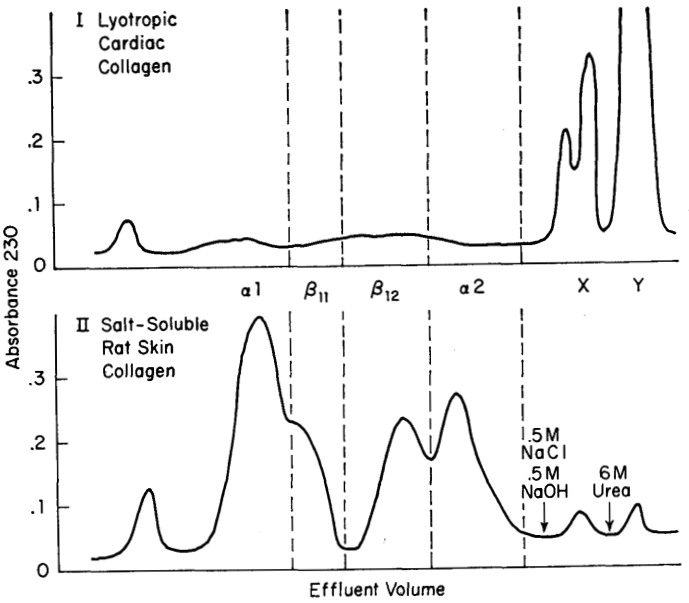

FIG. 2. Carboxymethyl cellulose chromatograms. I = bovine lyotropic cardiac collagen, II = salt-soluble rat skin tropocollagen. $\alpha 1$ and $\alpha 2$ = monomers, β_{11} and β_{12} = dimers, *X* and *Y* = higher molecular weight aggregates.

It can be seen that the yields of acid-soluble tropocollagen from rat skin and tendon represent 20 and 17% of the total collagen while only 2% of the rat cardiac collagen and less than 0.1% of the bovine cardiac collagen were solubilized. Reductions in solubility were also observed in the salt-soluble tropocollagen fractions. These data indicate a much more extensive interchain cross-linking network in the cardiac collagen than exists in either skin, tendon or striated muscle.

The disc gel electrophoresis patterns obtained from rat skin, bovine and rat cardiac acid-soluble tropocollagen are shown in Fig. 1. It is apparent that very little of the typical monomeric α-chains or dimeric β-components were present in the cardiac tropocollagen. Conversely, large quantities of γ-components and higher molecular weight aggregates were in evidence.

Figure 2 illustrates a typical carboxymethyl cellulose chromatogram obtained from rat skin acid-soluble tropocollagen and bovine lyotropic cardiac collagen. Similar patterns were also obtained when guanidine-soluble cardiac collagen was chromatographed. Here again it is apparent that cardiac collagen extracts were almost entirely devoid of the typically observed α- and β-components, while large quantities of higher aggregates were eluted with 0.5 *M* NaCl–0.5 *M* NaOH and 6.0 *M* urea. These results strongly suggest that cardiac collagen is virtually totally intermolecularly cross-linked.

Summary. Utilizing an improved isolation procedure, interstitial connective tissue from cardiac muscle was isolated in yields of approximately 6% of the dry muscle mass. The amino acid composition of intact cardiac collagen was markedly different from that of skin collagen. Yields of acid-soluble tropocollagen from bovine and rat cardiac collagen were only 0.1 to 1% of that obtained from rat skin collagen. Disc gel patterns and carboxymethyl cellulose chromatograms of soluble cardiac collagen revealed a marked reduction in α- and β-components and large quantities of higher molecular weight aggregates. These results suggest the presence of an extensive interchain cross-linking network in cardiac collagen.

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