Amino Acid Composition of Bovine Heart Valve Collagen[1] (36585)

R. I. BASHEY, Y. MORI,[2] AND L. PROSKY

Department of Medicine, Hahnemann Medical College and Hospital, Philadelphia, Pennsylvania 19102; and Division of Nutrition, Food and Drug Administration, Washington, D. C. 20204

These studies are part of an effort to determine if a relationship exists between changes in valvular collagen, acid mucopolysaccharides and vegetative endocarditis. Previous reports from this laboratory have shown hyaluronic acid to be a major component of the acid mucopolysaccharides in human heart valves (1, 2). We are unable to relate this finding to collagen composition because of difficulty in obtaining human valves in sufficient amounts for the isolation and purification of collagen. Therefore bovine heart valves were used for studies of biosynthesis of collagen and mucopolysaccharides (3–5). In this report we are presenting for the first time the amino acid composition of the purified bovine heart valve collagen and noting its possible significance.

Materials and Methods. Bovine hearts (3–4-year-old cows) were obtained from the slaughterhouse and brought to the laboratory packed in ice. The valves were carefully dissected free of muscle tissue. All valves (aortic, pulmonary, mitral and tricuspid) were rinsed several times with cold 0.9% NaCl solution. The valves were then minced and homogenized with cold 1 *M* NaCl in 0.02 *M* Tris buffer (pH 7.4) in a Virtis homogenizer and were further extracted separately and sequentially with 1 *M* NaCl, 0.75 *M* citrate buffer (pH 3.4) and 0.5 *M* acetic acid to remove the small amount of soluble collagen and noncollagen proteins. After the above treatment, the residue was washed with distilled water and lyophilized. The insoluble residue was divided in two parts. The first portion was extracted with 5% NaOH in saturated sodium sulfate for 5 days at 20° according to the method of Kuhn *et al.* (6). Only a small amount (less than 5%) of the insoluble collagen became citrate extractable after such a treatment. This finding again verified the fact that valvular collagen is extremely insoluble. The citrate-soluble collagen obtained from this procedure was relatively pure (hydroxyproline, 13.25%; low content of tyrosine, 0.51%).

The second portion of the lyophilized residue was gelatinized and purified by a modified Gross procedure [cited in (7)]. This highly purified gelatin was then lyophilized and had a low tyrosine content of about 0.78% and hydroxyproline content of 13.8%. Amino acid analyses were carried out on an automatic Model 120-B Spinco Amino Acid Analyzer following hydrolysis of the purified gelatin and citrate-soluble collagen with 6 *N* HCl for 16 hr at 110° in a sealed tube. The data on insoluble collagen represent an average of two determinations. No corrections were made for the destruction of amino acids during acid hydrolysis.

Results and Discussion. Table I shows the amino acid composition of insoluble and citrate-soluble collagen of bovine heart valves. Also included in this table is the amino acid composition of bovine skin gelatin. The analysis shows the distribution of amino acids present in valvular collagen to be characteristic and quite identical to that from other sources such as skin with the two exceptions: a lower content of methionine and a higher content of hydroxylysine in valvular collagen.

Little significance can be placed on the

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[2] Present address: Dept. of Biochemistry, Tokyo College of Pharmacy, Tokyo.

TABLE I. Amino Acid Composition of Bovine Heart Valve Collagens.

Amino acid	Citrate-soluble collagen	Insoluble collagen gelatin[a]	Ox skin gelatin[b]
	Residues/1000 residues		
Aspartic acid	43.6	41.9	46.0
Threonine	14.3	12.2	16.9
Serine	29.7	31.8	36.5
Glutamic acid	72.9	72.2	70.7
Proline	109.0	115.5	129.0
Glycine	370.0	347.8	333.0
Alanine	117.0	106.1	112.0
Valine	20.9	20.9	20.1
Half Cysteine	—	—	—
Methionine	0.7	1.2	5.5
Isoleucine	12.3	13.1	12.0
Leucine	23.7	24.5	23.1
Tyrosine	trace	0.4	1.5
Phenylalanine	11.0	12.3	12.3
Lysine	22.0	22.3	27.8
Arginine	44.1	49.2	46.2
Hydroxyproline	104.3	102.0	97.6
Hydroxylysine	14.7	12.8	5.5
Histidine	4.6	4.1	4.5

[a] Av of hydrolysates from 2 specimens.
[b] Eastoe, J. E., Biochem. J. **61**, 589 (1955).

relatively low methionine content reported for these heart valves since this amino acid may have been partially destroyed during the hydrolysis of the protein.

Hydroxylysine residues in collagen are synthesized from the parent amino acid lysine and have been shown to be linked to sugar residues in collagen and may be involved in the stability of the network of mature collagen fibrils. Butler and Cunningham (8) and also Spiro (9) have isolated hydroxylysine linked with mono- and disaccharides from alkaline collagen hydrolysates of various tissues. Steven and Jackson (10) have shown an increase in hydroxylysine residues in polymerized insoluble collagen samples. This polymerized collagen showed two amino acid peaks for hydroxylysine representing both *n*- and alo-isomers. Similarly, two peaks were obtained for hydroxylysine in both of our citrate-soluble and insoluble valve collagen preparations. Thus from the above, it becomes important to relate our findings of increased hydroxylysine residues in heart valve collagen to the specific function and stability of collagen in the valves and in further studies to correlate these changes with various disease states involving the heart valves.

Recently a new application of the cyanogen bromide technique for the preparation of peptides from soluble collagen (11) has been extended to that of insoluble collagen (12). Preparation of heart valve collagen utilizing this method will help us to further characterize this protein. A definite relationship between the elevated hydroxylysine content of heart valve collagen and the relatively insolubility of this collagen has not been established but will serve as a basis of speculation in future experiments.

Summary. Insoluble and citrate-soluble collagen from bovine heart valves were purified and analyzed for their amino acid composition. The data showed that the distribution of amino acids in this collagen is characteristic and identical to that from other sources except that hydroxylysine is present in significantly high amounts.

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