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DIFFERENCES IN VALYL-PROLINE SEQUENCE CONTENT IN ELASTINS FROM VARIOUS BOVINE TISSUES

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SUMMARY: A four-fold difference in the number of valyl-proline sequences recovered from bovine ligamentum nuchae elastin and ear cartilage elastin indicates that there are significant primary sequence differences between the two types of elastin. These results support our previous suggestion that the elastin associated with elastic cartilage is a different genetic type of elastin.

INTRODUCTION: Recently our laboratory has identified what appears to be a second genetic type of elastin from elastic cartilages (1). This elastin, which we have tentatively called "Type II" elastin, was isolated from the cartilage of bovine ear, epiglottis and larynx, using a nondegradative technique (2). Its amino acid composition was consistently similar and significantly different from elastin isolated from the usual, familiar sources such as bovine aorta, ligamentum nuchae and lung parenchyma and pleura.

Work is in progress to establish, by rigorous biochemical means, whether ear cartilage elastin is genetically distinct from ligamentum nuchae elastin. The availability of a simple and rapid method to isolate and quantitate valyl-proline (Val-Pro) sequences in elastin (3) has provided a means of investigating the occurrence of this sequence in the elastin molecule.

METHODS: Materials - Purified collagenase (type III fraction A), α-casein, α-chymotrypsinogen, pepsinogen (grade 1 from hog stomach), ribonuclease and trypsinogen (type 1A from bovine pancreas) were obtained from Sigma Chemical Co., St. Louis, Missouri. All other chemicals used were of reagent grade. Bovine specimens of young animals were obtained from a local slaughterhouse.

Preparation of purified elastin - Elastin was isolated using methods developed in our laboratory (2) and subsequently modified. In brief, lipids, collagen, structural glycoproteins and microfibrillar proteins were removed without significant degradation of the elastin or of its major crosslinks, desmosine and isodesmosine.

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Direct measurement of valyl-proline dipeptide - Elastin samples (10 mg) were hydrolyzed in alkali-resistant tubes with 1 ml 2N KOH under vacuum at 100°C for 22 h. Cooled hydrolysates were neutralized with concentrated $HClO_4$ using a trace of phenol red as an indicator. After cooling on ice for one hour, the samples were centrifuged (2000g, 10 min) to remove $KClO_4$. The clear yellow supernatant was chromatographed on a 60 x 0.9 cm column of cation exchange resin MR-205 at 55°C using a citrate gradient (pH 2.8, 0.2M Na^+ to pH 7.0, 0.75M Na^+). Val-Pro elutes after and close to phenylalanine, while the valyl-proline anhydride elutes at the front (3). Three separate preparations were hydrolyzed for each type of elastin.

Other investigators (3,4) have shown that in the elastins studied the sequence Val-Pro is preserved 50% as the dipeptide and 50% in the cyclized diketopiperazine form under similar alkaline hydrolysis conditions. In the present study Val-Pro is calculated taking into consideration the ninhydrin peak of Val-Pro eluting after phenylalanine. The calculation is based on a color factor obtained with an authentic sample of Val-Pro, which is 62% of the color factor of phenylalanine. The phenylalanine peak was used to calculate Val-Pro sequences/1000 amino acid residues as the recovery of this amino acid is similar after both acid and alkaline hydrolysis (5). After acid hydrolysis (6N HCl, 22 h, 105°C) the Val-Pro peak was shown to be composed of equimolar amounts of valine and proline.

RESULTS AND DISCUSSION: Representative amino acid compositions of ligamentum nuchae and ear cartilage elastins are shown in Table I. There are some consistent differences between the composition of cartilage elastin and that derived from ligamentum nuchae. Aspartic acid, serine, glutamic acid, tyrosine, lysine and arginine residues are increased while valine, alanine and isoleucine residues are decreased in elastin derived from cartilage. In this type of elastin the sum of isodesmosine and desmosine residues is less than that in the more familiar type of elastin (1).

Ligamentum nuchae elastin contains 115 residues of proline and 140 residues of valine, and assuming a random distribution of these amino acids in the molecule (3) the value for Val-Pro sequences would be 18 sequences/1000 amino acid residues (Table II). Ear cartilage elastin contains 132 residues of proline and 72 residues of valine and a similar calculation would give a value of 11 sequences/1000 amino acid residues. On the other hand in the 298 residues of ligamentum nuchae elastin so far accounted for (6) there are 14 Val-Pro sequences. On the assumption that the remaining unsequenced part of the molecule is similar there would be 41 Val-Pro sequences/1000 amino acid residues. No sequence data is available for ear cartilage elastin.

Table I

AMINO ACID COMPOSITION OF ELASTIN FROM VARIOUS BOVINE TISSUES*

Amino Acid	Ear	Ligamentum Nuchae
Hydroxyproline	14	9
Aspartic acid	41	7
Threonine	13	9
Serine	18	9
Glutamic acid	60	17
Proline	132	115
Glycine	309	312
Alanine	152	241
Valine	72	140
Methionine	T†	-
Isoleucine	11	26
Leucine	79	57
Tyrosine	26	8
Phenylalanine	35	30
Lysine	11	8
Histidine	1	1
Arginine	20	6
Isodesmosine	1.8	3.7
Desmosine	2.0	4.6
Lysinonorleucine	0.7	0.9

*Expressed as amino acid residues/1000 total amino acid residues.
†T, traces.

Table II

VAL-PRO SEQUENCE IN ELASTINS FROM BOVINE TISSUES*

	Ligamentum Nuchae	Ear Cartilage
Theoretical number based on random occurrences of Valine and Proline residues (3)	18	11
Estimated number based on available sequence data (6)	41	not available
Measured number uncorrected for recovery (see Methods)	19	5
Corrected range (see Results and Discussion)	34-79	9-21

*Expressed as Val-Pro sequences/1000 amino acid residues.

The data (Table III) on Val-Pro recovery from 5 proteins of known sequence shows that the recovery of Val-Pro sequences varies from about 24% to about 56% with an average of 38%. Our method directly detects 19 and 5 sequences of Val-Pro/1000 amino acid residues in ligamentum nuchae and ear cartilage elastins, respectively (Table II). The same results are obtained if the elastins are digested with elastase prior to alkaline hydrolysis. After correction based on the data of Table III, the number of sequences of Val-Pro in ligamentum nuchae

Table III

VAL-PRO RECOVERY FROM PROTEINS OF KNOWN SEQUENCE

	Actual Val-Pro dipeptides from amino acid sequence data* (7)	Recovered Val-Pro Dipeptides*	Recovery Factor
α Casein	25	6	24.0%
α Chymotrypsinogen	8	4	50.0%
Pepsinogen	9	5	55.6%
Ribonuclease	8	3	37.5%
Trypsinogen	4	1	25.0%
		average	38.4%

*Expressed as Val-Pro sequences/1000 amino acid residues.

elastin would be expected to range between 34 and 79 sequences/1000 amino acid residues. The value of 41 sequences extrapolated from the partial sequence data falls in this range and is clearly not consistent with a random distribution. The value for the Val-Pro sequence in ear cartilage elastin is calculated to be in the range of 9 to 21 sequences/1000 amino acid residues which is closer to a random distribution. One would also conclude that the sequence Val-Pro is about 4 times as frequent in the familiar elastin of ligamentum nuchae as in the elastin obtained from ear cartilage. This data adds weight to our preliminary conclusion (1) that significant sequence differences exist between the familiar elastin (Type I) and the newly discribed elastin from elastic cartilage. Hence, these latter elastins are probably different gene products which could be classified as Type II elastin.

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