HYDROXYLYSYL GLYCOSIDE CONTENT IN SKIN OF MICE: INFLUENCES OF AGING AND SEX ON HYDROXYLYSYL DISACCHARIDE

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ABSTRACT: Hydroxylysyl disaccharide in skin collagen of DDD strain mice was analyzed for the purpose of clarifying influences of aging and sex by column chromatography.

There were remarkable differences between hydroxylysyl disaccharide contents in skin collagen in DDD strain mice at 3 weeks of age and those at 5, 8, 12 weeks of age.

1) The contents were maximum at 3 weeks of age in both sexes. They rapidly decreased at 5 weeks of age and hardly changed thereafter.
2) The contents were less in female than in male at 3 weeks of age, whereas they were always more in female than in male at 5, 8, 12 weeks of age. There is an inverse relationship between those biological changes and morphological changes such as changes of collagen fibril size.

It is also suggested that those biological changes seem to precede morphological changes in some extent.

Physiological maturation and cross linking of collagen fibers, and the influence of sex hormones on collagen fibers in various tissues have been reported. Takeda et al.[1] found that the size of the collagen fibrils in the skin of DDD strain mouse showed significant sex differences after 5 weeks of age, a gradual increase with advancing age in males, no remarkable increase or decrease after 3 weeks of age in females and a significant thinness in the females. It is now generally accepted that size of the collagen fibrils increases in accordance with physiological maturation and relates to intermolecular cross link formation. This intermolecular cross link is said to be Schiff base formation between aldehyde residue of other molecules and amino acid residue of hydroxylysine (Hyl), to which galactose (Monosaccharide, M) or galactose-glucose (Disaccharide, D) are bound. Those hexoses are presumed to participate in regulation of collagen fiber formation or permeability of cell membrane.

The aim of our investigation was to assess the changes of Hyl-D or Hyl-M as related to changes in the size of collagen fibrils, according to sex and aging.

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MATERIALS AND METHODS

Twenty, healthy, normally fed female and male DDD strain mice, 3-12 weeks of age, were used. Animals were sacrificed under ether anaesthesia. The individual skin was dissected free of fat and hair and then minced in a Polytron homogenizer. Natural salt soluble (NSC), acid soluble (ASC) and insoluble collagen (ISC) were fractionated according to Heikkinen's method[2] and lyophilized. This dried collagen weighing 5 mg was sealed after dissolving with 1 ml of 2.5 N-NaOH. Hydrolysis was carried out at 110°C for 20 hours before centrifugation with 1 ml of 30% acetic acid. This supernatant was placed on the column (1.6×3.0 cm) of Amberlite CG-120 previously adjusted to H^+, type II. After neutralization, neutral and acidic amino acids were removed by 8% pyridine followed by extraction with 3 N-NH_4OH.

Amino acid analyses were carried out with a Hitachi KLA-5 analyzer after dissolving with 1.0 ml of citrate buffer adjusted to pH 2.2. As seen on the chromatography, peaks appeared within 80 minutes in the order of Hyl-D, Hyl-M and Hyl, these peaks were then integrated. Standard Hyl-hexose compounds were obtained from natural sponge and a hydrolysis was carried out with 2 N-NaOH. Chromatography was carried out on a column of Bio-Gel P-2 (1.6×100 cm), before the hexose contained fractions were checked using the Orcinol sulfate method. After those fractions were condensed, Hyl-hexose compounds were collected using a high voltage electrophoresis (CAMAG HVE system). Figures 1 and 2 show the elution profiles of standard Hyl-D and standard Hyl.

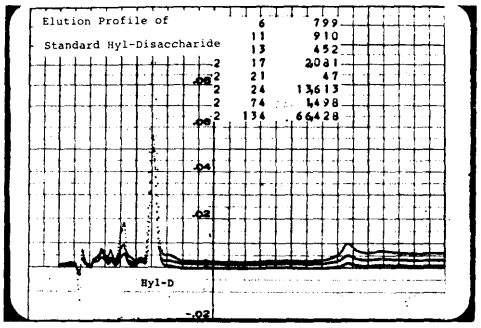

Fig. 1 Elution profile of standard Hyl-D.

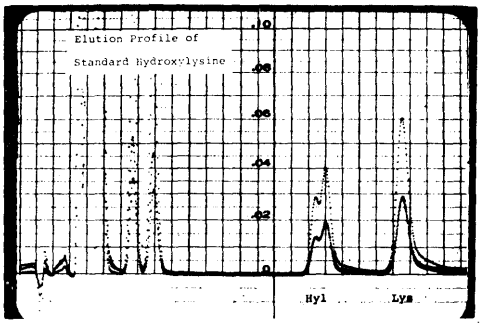

Fig. 2 Elution profile of standard Hyl.

RESULTS

Ratios of Hyl-D/Hyl were calculated in conformity with elution profiles of ISC and ASC fractions of skin collagen (Fig. 3, Table 1).

In the ISC fraction, ratios of Hyl-D/Hyl at 3 weeks of age were remarkably higher than

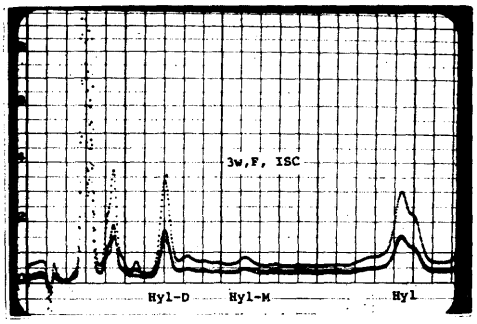

Fig. 3 Elution profile of ISC fraction of skin collagen in a 3-week-old female DDD strain mouse. Hyl-D/Hyl: 1.053

Table 1 Ratios of Hydroxylysyl Disaccharide/ Hydroxylysine in Collagen in Skin of DDD Strain Mice

		3 W	5 W	8 W	12 W
ISC	M	1, 245	0, 588	0, 535	0, 278
	F	1, 053	0, 678	0, 610	0, 779
ASC	M	1, 637	0, 329	0, 512	0, 235
	F	0, 667	0, 691	0, 594	0, 633
NSC	M	0, 305	0, 173	0, 692	0. 910
	F	0, 418	0, 651	—	—

those at 5, 8, 12 weeks of age, in both sexes. The ratios were larger in females after 5 weeks of age, less in males at 3 weeks of age and tended to decrease in males with advancing age.

In the ASC fraction, ratios of Hyl-D/Hyl at 3 weeks of age were much greater than those at 5, 8, 12 weeks of age in male, although little change was seen in the females. The ratios were less in females at 3 weeks of age, whereas they were higher in females after 5 weeks of age, in both the ASC and ISC fractions.

DISCUSSION

Spiro[3,4] demonstrated that collagen is a kind of glycoproteins because hexoses, Gal or Gal-Glc bind to Hyl in the collagen molecule. Thus, it is possible to clarify the specificity of the collagen by analysing those hexoses. The relationship between the hexose and morphological changes of collagen fiber[5,6] or aging[7] has been reported by other workers. Takeda et al.[1] reported that morphological changes of skin of DDD strain mouse, as seen under the electron microscope were influenced by aging and sex hormones.

It is generally accepted that oxidation of lysine to Hyl takes place inside the cell, followed by glycosylation and cross-link formation outside the cell. Morgan et al.[8] demonstrated that the amino acid sequence around hexose-Hyl is Gly-X-Hyl (Gal-Glc)-Gly-Y-Arg. Hexose transferases, which are included in the cell membrane have such a high specificity that they discriminate specific Hyl. Bosmann and Eylar[9,10] reported that this two-stage reaction is actually the binding of Gal or Glc in collagen. After the collagen fiber subjected to glycosylation is secreted outside the cell, intramolecular or an intermolecular cross-linking occurs.

Maturation of collagen is thus assumed to be a morphological increase in size of the collagen fibrils. There is a general consensus that an increase in size of the collagen fibrils is made by direct binding of the collagen molecule to the surface of this fiber. The collagen molecule does in itself have a certain regarding specificity organs and tissues, whereas interstitial high or low molecules participate in regulation of collagen fibril size. Grant et al.[5] compared amino acid composition or hexose composition of polymeric collagen in connection with collagen fibril size extracted from human and bovine tissues. These workers found that hexose content varied considerably and decreased in inverse proportion to the increase in size of the collagen fibrils. We found a remarkable difference in the Hyl-D of skin collagen of 3 week-old mice as compared to 5–12 week-old mice. An inverse relationship was seen in the skin collagen of DDD strain mouse. Sex differences regarding the hexose content were clearly revealed. The hexose content decreased with advancing age in males, yet there was little variation in the females.

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