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HYDROXYPROLINE TO HYDROXYLYSINE MOLAR RATIO INDICATES COLLAGEN TYPE

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Abstract. By using the molar ratio Hyp to Hyl, types I and II of collagen can be differentiated in 0.5 to 10 mg of dried, defatted tissue. Analyses on human skin, tendon, bone, aorta, cartilage, as well as nucleus pulposus, and annulus fibrosus of intervertebral discs are reported. Analyses of collagen of mesenchymal tissues of other vertebrates are also reported. From amino acid analysis of purified collagen samples published in the literature, the molar ratio Hyp/Hyl was calculated. Type I and type III collagen were differentiated from type II, and the latter was differentiated from type IV collagen. The molar ratios obtained with our analyses followed closely the values from previously reported amino acid analyses on purified collagen.

Key words: Hydroxyproline/Hydroxylysine molar ratio; Collagen typing

Four different types of collagen have been isolated from tissues of vertebrates (19). In type I, present in skin, tendon, bone, and dentine, the collagen monomer (tropocollagen) is composed of two identical α_1 chains and one α_2 chain $[\alpha_1(I)]_2\alpha_2$, while the other three types have three identical α_1 chains (18). The tropocollagen of cartilage (Type II) is symbolized by $[\alpha_1(II)]_3$, while fetal skin, adult skin (19), aorta, and uterus contains type III as represented by $[\alpha_1(III)]_3$ and that of basement membranes, type IV $[\alpha_1(IV)]_3$.

As type II collagen is known to contain more hydroxylysine (Hyl) than type I, with practically constant hydroxyproline (Hyp), expressed as residues per 1 000 amino acid residues, we considered the possibility of using the molar ratio Hyp to Hyl as a parameter of the collagen types. This ratio might be particularly useful in characterizing type IV collagen, which, due to a very high content of Hyl with a smaller amount of Hyp, gives a ratio which is even lower than type II collagen. The classification of the collagen types (18) has hitherto been based on isolation of collagen, cyanogen bromide cleavage at methionyl residues, isolation of the peptides cleaved, and amino acid analysis of the fragments resulting from the treatment. The amount of tissue required to perform such studies is quite considerable.

In addition to calculating the Hyp/Hyl ratios from the amino acid analyses of isolated native collagen α_1 and α_2 chains as well as their dimers reported by other authors (20) we determined the same ratio in our own analysis of small quantities of human skin and other mesenchymal tissues.

MATERIAL AND METHODS

Cylindrical biopsies, 2–4 mm, of human skin were taken with a stainless steel punch instrument (4). Necropsies of human skin, aorta, cartilage, bone, tendon, as well as annulus fibrosus and nucleus pulposus of intervertebral discs, were removed at the autopsy of 3 traffic accident victims in their thirties (5). Samples of skin, cartilage, and bone of other mammals, viz. seal, rat and mouse, as well as birds and fishes, were also analysed. A total of 247 samples were studied.

The tissues were treated with three changes of acetone per day for 3 days. On the 4th day, they were treated with a mixture of equal parts of acetone and ether (3 changes). On the 5th day, they were treated with one change of ether. The ether was evaporated, and the samples were placed in a stainless steel desiccator (Nikortanks) for 3 days (i.e. to constant weight). The samples were weighed in a Metler ultramicro balance. The final weight of the dried and defatted samples varied between 0.5 and 10 mg. Then the samples were hydrolysed in 6 N HCl at 116°C for 16 hours and, after hydrolysis, each sample was divided into two parts. The two aliquots were

Table I. *Molar ratio Hyp/Hyl as calculated from analyses of collagen in a variety of tissues from various vertebrates*

	No. of samples	Collagen type	Molar ratio Hyp/Hyl
Man			
Skin	138	I+III	16.5 ±0.5
Tendon	1	I	15.7
Bone	4	I	22.1 ±0.5
Aorta thoracica	2		14.7 ±2.7
Annulus fibrosus (5)	35	I+II	9.45±1.27
Outer	17		10.3 ±1.0
Inner	18		8.64±0.8
Nucleus pulposus (5)	16	II	7.77±0.8
Cartilage	4	II	7.4 ±0.2
Seal			
Skin	2		18.9 ±0.3
Rat			
Skin, ventral	4	I+III	15.5 ±0.6
Skin, dorsal	4	I+III	16.5 ±0.4
Tendon, tail	2	I	11.0 ±0.7
Cartilage	2	II	6.9 ±0.2
Mouse			
Skin, ventral	6	I+III	19.0 ±0.4
Skin, dorsal	6	I+III	18.0 ±0.6
Skin, tail	2	I+III	15.2 ±1.2
Tendon	2	I	15.2 ±1.0
Birds:			
Chicken			
Skin	4	I+III	14.8 ±0.3
Tendon	2	I	13.8 ±0.4
Calvaria	2	I	16.0 ±0.3
Cartilage	2	II	4.8 ±0.2
Penguin			
Skin	1	I	20.0
Fishes:			
Trigla gurnardus			
Skin	1		14.5
Swim bladder	1		7.5
Rays	1		11.6
Dorsal fin	1		5.4
Gadus morrhua			
Skin	1		10.9
Bone	1		7.6

evaporated separately (4) and analysed for Hyp and Hyl, using the automated procedures of Blumenkrantz & Asboe-Hansen (2, 3).

From amino acid analyses of purified collagen of different tissues reported by various authors, the molar ratio Hyp/Hyl was obtained.

Calculations

Results were expressed as μg Hyp or Hyl per 10 mg dried and defatted tissue (DDT), and the Hyp/Hyl molar ratio was calculated. The ratios were compared and differences evaluated by using Student's *t*-test.

RESULTS

In Table I, the molar ratios Hyp/Hyl of the samples analysed are presented. The ratios of samples of human skin, tendon, bone, aorta, cartilage, annulus fibrosus (AF), and nucleus pulposus (NP) are presented, together with a similar analysis of tissues of other vertebrates. The differences between the molar ratios Hyp/Hyl of skin, bone and tendon were found statistically non-significant, while the difference between the ratios of the mentioned tissues and cartilage was significant ($P<0.01$).

The difference in Hyp/Hyl molar ratio between AF and NP was statistically significant ($P<0.01$). On comparison of human cartilage with NP ratios, the difference was statistically non-significant ($P>0.05$). The ratios of the aorta thoracica are also significantly different from that of cartilage ($P<0.05$).

The ratios calculated on the basis of the amino acid analyses of various tissues performed by other authors are given in Table II. The molar ratios Hyp/Hyl of types I and III collagen were significantly higher than those of types II and IV. This finding is attributable to their lower content of Hyl in relation to Hyp. It was found in our own analyses, performed on dried and defatted tissue, as well as by calculation from the amino acid analyses of purified collagen.

DISCUSSION

Normal cartilage produces only type II collagen in culture, whereas slices of osteoarthritic cartilage also synthesize type I (25). The fact that the polypeptides isolated from urine of patients with Paget's disease show the same Hyp/Hyl ratio as normal human bone (Table II) confirms the notion that the polypeptides mentioned originate from the remodelling of bone which takes place in this disease (15). The molar ratio Hyp/Hyl in urine may thus be a useful indicator of collagen resorption, breakdown, or synthesis of incompletely cross-linked collagen.

Although the collagen of skin, tendon, bone, and

Table II. *Molar ratio Hyp/Hyl as calculated from amino acid analysis performed by various authors on purified collagen and subunits*

R=Rachitic, NR=non-rachitic

Tissue/organ	Vertebrate	Isolated collagen	α_1	β_{11}	β_{12}	α_2	β_{22}	Collagen type	Ratio	Author
Tendon (tail)	Rat	13.6	19.3	22.0	11.7	8.2				Piez et al. (20)
Tendon	Man	14.0								Piez & Linkins (21)
Tendon	Dog	17.0								Kefalides (14)
Skin	Man	16.0	20.0	20.0	13.4	10.8	10.3			Bornstein & Piez (6)
Skin	Rat	16.1	22.3	24.8	14.1	10.7				Piez et al. (20)
Skin	Calf (3 mo. old)							I	11.1	Bailey & Sims (1)
Skin	Calf (3 mo. old)							III	17.0	Bailey & Sims (1)
Skin	Calf (9 mo. old)							I	13.3	Bailey & Sims (1)
Skin	Calf (9 mo. old)							III	19.7	Bailey & Sims (1)
Skin	Chick							I	15.36	Trelstad et al. (25)
Skin	Lung-fish	13.7								Eastoe (7)
Liver	Man							I	18.5	Rojkind & Palomo (22)
Liver	Man							III	22.6	Rojkind & Palomo (22)
Bone	Man	19.6								Krane et al. (15)
Bone (diaphysis)	Chick(R)		10.6							Toole et al. (24)
Bone (diaphysis)	Chick(NR)		15.7							Toole et al. (24)
Bone (diaphysis)	Chick(R)					9.2				Toole et al. (24)
Bone (diaphysis)	Chick(NR)					12.4				Toole et al. (24)
Bone	Chick		18.5							Miller et al. (18, 19)
Bone	Chick					12.5				Miller et al. (18, 19)
Cartilage	Chick	4.5								Trelstad et al. (25)
Cartilage (epiphysis)	Chick(R)	4.8								Toole et al. (24)
Cartilage (epiphysis)	Chick(NR)	5.4								Toole et al. (24)
Cartilage (growth plate)	Chick(R)	4.4								Toole et al. (24)
Cartilage (growth plate)	Chick(NR)	4.5								Toole et al. (24)
Cartilage (nasal)	Ox	4.5								Lee-Own & Anderson (17)
Cartilage (larynx; hyaline)	Pig							II	4.3	Eyre & Muir (9)
Cartilage (ear; elastic)	Pig							II	5.3	Eyre & Muir (9)
Cartilage (meniscus; fibrous)	Pig							I	10.2	Eyre & Muir (9)
Cartilage	Chick							II	4.5	Lane & Weiss (16)
Cartilage (larynx)	Pig	4.7								Eyre & Muir (8)
Annulus fibrosus	Pig	6.6								Eyre & Muir (8)
Nucleus pulposus	Pig	4.3								Eyre & Muir (8)
Basement membrane (glomeruli)	Ox							IV	2.7	Sato et al. (23)
Basement membrane (glomeruli)	Ox							IV	2.8	Sato et al. (23)
Basement membrane	Man							IV	3.0	Sato et al. (23)
Basement membrane (tubule)	Man							IV	2.8	Sato et al. (23)
Basement membrane (glomeruli)	Man							IV	2.65	Kefalides (12)
Basement membrane (glomeruli)	Man							IV	3.1	Kefalides (13)
Basement membrane (lens capsule)								IV	3.0	Kefalides & Denduchis (11)

Table II. *(Cont.)*

Tissue/organ	Vertebrate	Isolated collagen	α_1	β_{11}	β_{12}	α_2	β_{22}	Collagen type	Ratio	Author
Basement membrane (Descemet's)	Dog							IV	4.1	Kefalides & Denduchis (11)
Basement membrane (lens capsule)	Sheep							IV	2.3	Kefalides (12)
Basement membrane (lens capsule)	Sheep							IV	2.58	Gelman et al. (10)
Basement membrane (lens capsule)	Man							IV	3.0	Kefalides (12)

dentine contains $[\alpha_1(I)]$ plus α_2 chains, while cartilage contains only α_1 chains $[\alpha_1(II)]_3$, the latter α_1 chains can be differentiated from the former by their higher content of Hyl. The same is true of type IV basement membrane collagen containing $[\alpha_1(IV)]_3$ chains, which are particularly rich in Hyl.

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