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Hyaluronidase in human somatic tissues and urine: polymorphism and the activity in diseases

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The polymorphism of hyaluronidase (EC 3.2.1.35) (Hyase) was studied on a hyaluronan-polyacrylamide gel. Liver, placenta, ovary and breast tissue were found to have 7 active isoforms while leukocytes and platelets 5 and fibroblasts displayed no hyaluronidase activity. In serum, synovial fluid and urine soluble the most acidic forms are present.

Desialylation showed that most of the hyaluronidase isoforms differ in the content of sialic acid.

In patients with rheumatoid arthritis, hyaluronidase activity in the synovial fluid varied from not detectable to very high. A partial deficiency was demnostrated in sera from some patients with dysostosis multiplex without mucopolysaccharidurie. In I-cell disease, hyaluronidase activity in serum was as that in controls.

Duran-Reynals has discovered that testicular extracts contain a factor that enhances the spreading of viral agents [1] and he called it the spreading factor. It was then further characterized as a hyaluronan-degrading enzyme and called hyaluronidase (EC 3.2.1.35) by Chain & Duthie [2]. Another hyaluronidase was also shown to be present in human serum and synovial fluid and rat plasma and urine but, unlike the testicular hyaluronidase, this somatic enzyme had an acidic pH *in vitro* [3–5]. Although testicular and somatic hyaluronidase have similar substrate specificity, they differ in immunological, physical and kinetic properties. However, the characteristics of somatic hyaluronidases active in lysosomes, plasma, synovial fluid and tissues *in vivo* are still unknown.

Hyaluronidases degrade not only hyaluronan (hyaluronic acid), a major component of the extracellular matrix, but also chondroitin 4-sulfate and chondroitin 6-sulfate.

The hyaluronan-hyaluronan synthetase-hyaluronidase system plays a major role in many biological processes involving the maintenance and integrity of the extracellular matrix, and its interactions with the cell surface [6]. These processes include phagocytosis, cell adhesion, differentiation, endothelial cell morphogenesis [7], embryonic morphogenesis and organogenesis [8], normal and tumour cell proliferation [9]. Not only hyaluronidase but also hyaluronan is present in serum, lymph [10],

interstitial fluid [11], bone [12] and cerebral white and gray matter [13].

In a previous work, in which hyaluronan-polyacrylamide gel electrophoresis was used to study hyaluronidase activity [4] in the sera of various species, we found that this enzyme displayed in each species a specific pattern [14].

This prompted the present study of the multiple forms and activity of hyaluronidase in human somatic tissues and body fluids. In search for tissue-specific hyaluronidase patterns, we analyzed by electrophoresis samples of liver, ovary, breast, placenta, platelets, leukocytes, serum, synovial fluids and urine.

MATERIALS AND METHODS

Electrophoresis [4] was performed on a hyaluronan-containing polyacrylamide gel that visualizes hyaluronidase activity when incubated at pH 3.5 and stained with "stains all" (Kodak). The different bands were numbered in the order of decreasing anodic mobility.

RESULTS AND DISCUSSION

In serum and synovial fluid only the most acidic and soluble forms were present called Hyases (1, 2 and 3). Liver and placenta were found to have at least 7 active forms; in ovary and breast tissue the same forms were present but they were less active. Leukocytes and platelets had no Hyase 1 or Hyase 2. Fibroblasts displayed no hyaluronidase activity at all. The presence of the more acidic forms in liver, placenta and other tissues as well, suggests that they might be located in the cytosol (Hyase 3), ready for secretion, and in the extracellular space.

Treatment of hyaluronidase with sialidase showed that the different forms of hyaluronidase contained different amounts of sialic acid. Desialylated serum hyaluronidase was less soluble but more active than the sialylated enzyme. This raises the question of whether, *in vivo*, the existence of different forms is connected with cell processing and secretion of hyaluronidase. Human hyaluronidase in somatic tissues, serum, synovial fluid and urine seems to be a single glycoprotein exhibiting different degrees of glycosylation and containing different amounts of sialic acid.

The hyaluronidase activity in the sera of 62 randomly chosen breast cancer patients was unchanged compared to that of the controls. Out of 28 randomly chosen patients with rheumatoid arthritis of variable duration, in 13 the hyaluronidase activity in the synovial fluid was changed (Table 1). In 6 of those 13 patients, hyaluronidase activity was changed also in their serum but less markedly.

A partial deficiency of the enzyme was demonstrated in some patients displaying dysostosis multiplex, without mucopolysaccharidurie. Those patients, all of them children between 6 and 10 years old, displayed various bone disturbances, vertebral malformations, shortening and thickening of long bones, joint deformities; three of them displayed dwarfism; visceromegaly as well as gross mental retardation were absent. The activities in serum of β-*N*-acetylglucosaminidase and β-glucuronidase (enzymes participating in the total degradation of hyaluronan) and the serum hyaluronan level were the same as in controls. After one year three patients were still deficient

Table 1
Hyaluronidase activity in the synovial fluid and serum of patients with rheumatoid arthritis

Intensity of hyaluronidase activity (nos. of patients in parentheses)	
Synovial fluid (6.7 µl per slot)	Serum (0.5 µl per slot)
Extremely high (1)	ND
High (1)	high
High (1)	N
Moderate (15)	N
Weak (3)	N
Weak (1)	weak
Very weak (4)	ND (1) weak (3)
Undetectable (2)	ND (1) weak (1)

N, values as in control serum; ND, not determined. Numbers of sera in the right side column are the same as in the left side column, unless otherwise indicated.

in hyaluronidase but after five years of treatment the enzyme activity became normal.

In one case of Sanfilippo A disease the hyaluronidase activity in serum was decreased and the seric hyaluronan concentration was 5 times higher than that of the controls. In two cases of I-cell disease, one had a slightly increased hyaluronidase activity, the other displayed a hyaluronidase deficiency associated with an increased hyaluronan level in serum. This last finding suggests that hyaluronidase activity in serum is not dependent on mannose-6-phosphate receptors.

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