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THE ENZYMATIC ACTIVITY OF SPIDER VENOM

On the influence of sulfonated polysaccharides on the proteolytic and hyaluronic acid splitting activity of spider venom

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In a previous communication we discussed the proteolytic activity ot the venom of *Lycosa raptoria* and *Ctenus nigriventer*. When casein is employed as substrate the pH optima of the proteolytic enzyme are pH 7,5 and pH 8,0 for the venom of *Lycosa raptoria* and *Ctenus nigriventer* respectively. Calculation of the proteolytic activity in respect to crystalline trypsin (Armour) gives an amount of 3 — 4 crystalline trypsin for one milligramm of the venom (Kaiser, 13, 14). The negative results of other authors which were not able to detect proteolytic enzymes in spider venom (Vellard, 21, 22) may be caused by their method employed whose accuracy is not enough to detect minimal amounts of proteolysis.

Different authors have investigated the inhibiting action of heparin, a negatively charged macromolecule, on tryptic proteolysis. Glazko and Ferguson (9) and Horwitt (10, 11, 12) showed that trypsin's activity is inhibited by heparin employing casein as substrate. Concentrations between 500 and 600 U. heparin are still active. Later Rocha e Silva and Andrade (19) and Wells et al. (23) demonstrated that crystalline trypsin is inhibited by heparin and that commercial trypsin preparations were not influenced. We have taken up this question again in this laboratory and demonstrated that heparin (liquemin Roche, heparin Vitrum) in concentrations from 62,5 — 1200? were without any influence on tryptic proteolysis. The employed crystalline trypsin and commercial trypsin preparations. Casein, fibrinogen, fibrin and gelatine served as substrates (14).

In this communication we will discuss the influence of heparin and sulfonated peptic acid on the proteolytic activity of spider venoms. Casein was employed as substrate.

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METHODS

The proteolytic activity was determined according to the method of Kunitz (15), modified in this laboratory (Pantlitschko, Kaiser and Andres, 18). This method is based on measuring the ultraviolet absorption of tyrosin and tryptophan freed by proteolytic enzymes from the substrate. There is a simple, linear correlation between extinction and enzyme concentration which makes it possible to calculate enzyme concentration and the inhibition of the enzymatic process.

The venoms of *Lycosa raptoria* and *Ctenus nigriventer* were tested. As inhibitory substances heparin (liquemin Roche, heparin Vitrum) and different preparations of sulfonated peptic acid were investigated. The average molecular weight of the sulfonated ranged from 20.000 — 90.000. Heparin (62,5 — 1200) nor sulfonated peptic acid (100 — 2000) showed any inhibitory action on the proteolytic of spider venom. The proteinase of spider venom behaves in this respect like crystalline trypsin from beef pancreas.

RESULTS

Besides the described proteolytic enzyme in spider venom, we were able to detect a considerable amount of an enzyme splitting hyaluronic acid (arachnomucinase). The occurrence of this hyaluronic acid splitting enzyme (hyaluronidase) in animal organs and different bacterial strains is well known Meyer (16), Gibian (8). Also the venom of insects and of different snakes contains different amunts of hyaluronidase, a fact pointed out by different authors. (Duran — Reynals (3,4), Tabarini — Castellani (20), Eichbaum (5), Zeller (25), Werle et al. (24), Chain and Duthrie (1), Farilli (6), de Marco (2,), etc.).

We were able to show in this laboratory that strongly negative charged chain molecules (sulphuric esters of polysaccharides) are potent inhibitors of hyaluronidase in relatively small concentrations. Negatively charged spheric molecules are without any effect. Positively charged macromolecules (histone, protamine, peptone) are activators of the breakdown of hyaluronic acid by hyaluronidase (Pantlischko, Kaiser) (17).

We have investigated the action of negatively charged chain molecules on the activity of arachnomucinase. The activity was determined according to the method described in this laboratory (17). Heparin and sulfonated hyaluronic acid were investigated. Table 1 shows the results with heparin. Heparin is active up to a concentration of 5^{10}-^{5} A concentration of $2{,}5^{10}\text{-}^{4}$ gives a complete inhibition of arachnomunicinase. Between concentrations of 4^{10}-^{3} and $2{,}5^{10}\text{-}^{4}$ there exists a nearly linear correlation between the inhibition of arachnomucinase's activity and the concentration of heparin. The same results

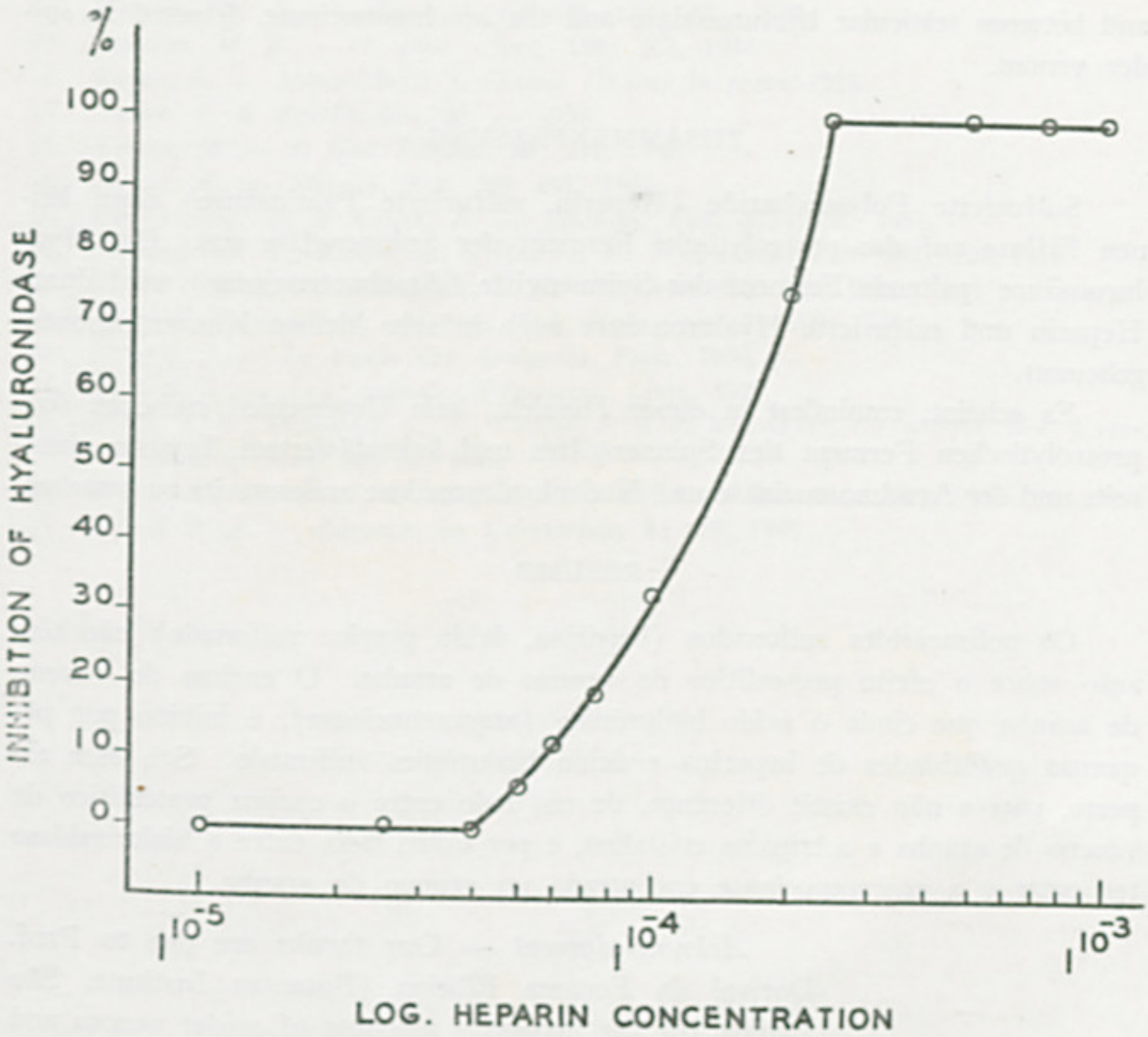

TABLE 1

Inhibition of arachnomucinase by different concentrations of heparin (Method: Pantlitschko, Kaiser, 1951).

could be found with sulfonated hyaluronic acid. Testicular hyaluronidase is inhibited by similar concentrations. Basic substances like histone, protamine and peptone are activators of the arachnomucinase. It seems that at least in this respect there exists no difference between testicular hyaluronidase and arachnomucinase.

SUMMARY

There is no influence of sulfonated polysaccharides (heparin, sulfonated pectic acid) on the proteolytic activity of spider venom. The enzyme from spider venom splitting hyaluronic acid (arachnomucinase) is inhibited by heparin and sulfonated hyaluronic acid up to very small concentrations.

There seems to exist, at least in this respect, no difference between the proteolytic enzyme of spider venom and crystalline trypsin on the one hand

and between testicular hyaluronidase and the arachnomucinase detected in spider venom.

ZUSAMMENFASSUNG

Sulfurierte Polysaccharide (Heparin, sulfurierte Pektinsäuse) üben keinen Eifluss auf das proteolytische Ferment der Spinnengifte aus. Das Hyaluronsäure spaltende Ferment der Spinnengifte (Arachnomucinase) wird durch Heparin und sulfurierte Hyaluronsäure noch in sehs kleinen Konzentrationem gehemmt.

Es scheint, zumindest in dieser Hinsicht, kein Unterschied zwischen dem proteolytischen Ferment des Spinnengiftes und kristallisiertem Trypsin einerseits und der Arachnomucinase und Hodenhyaluronidase andererseits zu bestehen.

RESUMO

Os polisacárides sulfonados (heparina, ácido peptico sulfonado) não têm ação sobre o efeito proteolítico do veneno de aranha. O enzima do veneno de aranha que cinde o ácido hialurônico (aracnomucinase), é inibido por pequenas quantidades de heparina e ácido hialurônico sulfonado. Sob êsse aspecto, parece não existir diferença, de um lado entre o enzima proteolítico de veneno de aranha e a tripsina cristalina, e por outro lado, entre a hialuronidase testicular e a aracnomucinase encontrada no veneno de aranha.

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