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PROTEOLYTIC MODIFICATION OF STAPHYLOKINASE

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Summary

There are three types of staphylokinase of different isoelectric points (6.7, 6.1 and 5.7). Staphylokinase of p*I* 6.7 was converted to that of p*I* 6.1 and then to that of p*I* 5.7 by the treatment with trypsin.

Heterogeneity of staphylokinase might be the result of post-translational modification by proteolytic enzyme.

The isolation of staphylokinase of different isoelectric points has been reported by several workers [1—4]. The minor differences between p*I* values observed in the reports were probably due to the difference of bacterial strains used in production.

Staphylokinases of p*I* 5.7, 6.1 and 6.7 were isolated from the culture supernatant in our experimental system. In the course of an experiment designed to elucidate the interaction between canine plasminogen and these three staphylokinases, a conversion of staphylokinase of p*I* 6.7 (staphylokinase 6.7) and staphylokinase of p*I* 6.1 (staphylokinase 6.1) to staphylokinase of p*I* 5.7 (staphylokinase 5.7) by treatment with canine plasmin was observed [4]. It was assumed from this evidence that staphylokinase 5.7 was a product of post-translational modification of staphylokinases 6.7 and 6.1 by the action of some proteolytic enzyme which might be present in the staphylococcal culture. However, the relationship between staphylokinase 6.1 and 6.7 has remained undetermined.

In this communication, an attempt is made to reveal the relationship of all three of these staphylokinases.

The identification of the three staphylokinases was carried out by electrophoresis on 7% polyacrylamide gel at pH 9.4 [5]. The migration rates were expressed as a ratio to human hemoglobin which was coelectrophoresed with samples. The migration rates of staphylokinases 0.6, 0.8 and 1.0 corresponded to p*I* 5.7, 6.1 and 6.7, respectively. The activity of staphylokinase

was measured by the fibrin agar plate method. 20 ml of molten agar (1.6%) were kept at 56°C containing 0.2% $CaCl_2 \cdot 2H_2O$, 10^{-3} M sodium azide and Tris·HCl buffer, pH 7.6, $5 \cdot 10^{-2}$ M. 1 ml of 0.5% bovine fibrinogen and 0.5 ml of human plasma were added to the mixture. After a 5-min incubation at 56°C, the mixture was poured into a plastic dish (15 × 10 cm). After solidification, wells with a diameter of 5 mm were made and 1-mm thick slices of the polyacrylamide gel on which staphylokinase were placed in them and were then filled with the buffer. After incubation at 37°C for 18 h, the diameter of the clear zone surrounding the well was measured. The diameter was proportional to the logarithm of staphylokinase concentrations. One unit of staphylokinase was defined as the amount which yields a clear zone with diameter of 10 mm. *Staphylococcus aureus* 959-76 was used which was isolated from a patient and produced staphylokinase 6.7 exclusively. Staphylokinase was partially purified by gel filtration on Sephadex G-75 and isoelectric focusing.

When staphylokinase 6.7 was treated with lyophilized bovine pancreatic trypsin (2.4 μg) and electrophoresed after adding soybean trypsin inhibitor (10 μg), rapid elimination of staphylokinase 6.7 and simultaneous appearance of staphylokinase 6.1 and 5.7 were observed (Fig. 1 (a) and (b)). By prolon-

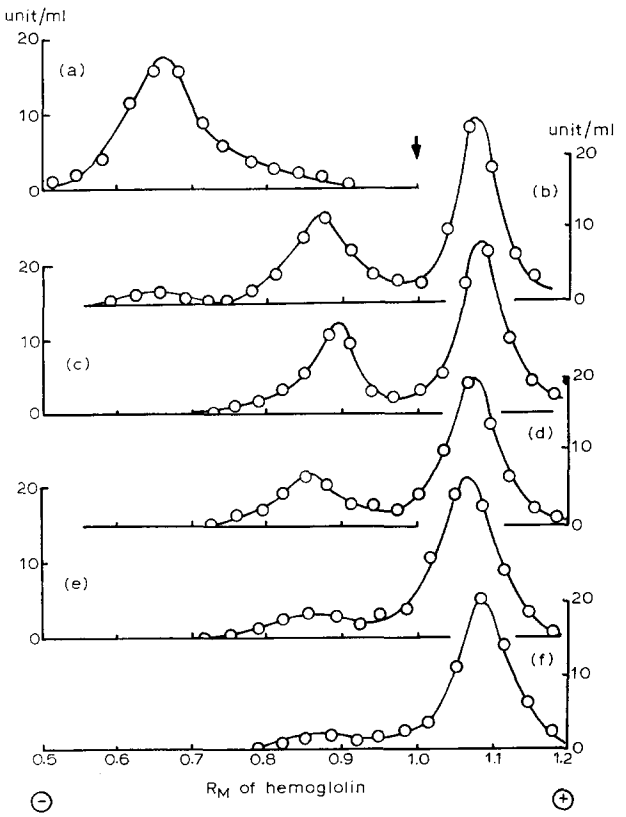

Fig. 1. Modification of staphylokinase by trypsin. 30 units of staphylokinase 6.7 were treated with trypsin at 37°C for 0 min (a), 2 min (b), 5 min (c), 15 min (d), 35 min (e), 75 min (f) and then electrophoresed. The arrow indicated the position of hemoglobin.

ged incubation with trypsin, decrease of staphylokinase 6.1 occurred sequentially and finally all the activity of staphylokinase was detected at the fraction corresponding to staphylokinase 5.7 (Fig. 1 (c)—(f)).

From the above-mentioned results, staphylokinase 6.7 was presumably converted to staphylokinase 6.1 and then to the 5.7 form. Although both proteolytic enzymes, plasmin and trypsin, hydrolyze peptide chains at the basic amino acid residue, plasmin did not react with staphylokinase 6.7 to produce staphylokinase 6.1 [4]. This evidence suggested that plasmin might not be accessible to the peptide bond which was contained in staphylokinase 6.7 and was susceptible only to trypsin for some reason, i.e. the molecular weight of plasmin (approx. 85 000) was much larger than that of trypsin (20 000).

The modification of staphylokinase molecule may be a minor one and does not affect its function, because these three staphylokinases have almost same activity.

The results also suggest that the structural gene of staphylokinase might be the only one which has the information of staphylokinase 6.7. Staphylokinase 5.7 and 6.1 might be the product of a post-translational modification of staphylokinase 6.7 by proteolytic enzyme.

It is necessary to isolate the proteolytic enzyme in vivo before reaching a final conclusion. Attempts to detect the proteolytic activity in culture supernatant or cell-free extract have however been unsuccessful at the present stage.

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