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Prothrombin Activation by a Metalloprotease from *Staphylococcus aureus*

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Formation of thrombin during incubation of purified bovine prothrombin with purified staphylococcal metalloprotease has been investigated. Thrombin activity was estimated by examination of clotting time and by digestion of a synthetic substrate, Chromozym TH. The metalloprotease caused direct activation of prothrombin which was inhibited by the addition of ethylenediaminetetraacetic acid. Metalloprotease produced by some strains of *Staphylococcus aureus* may simulate staphylocoagulase activity.

Conversion of prothrombin to thrombin may occur nonspecifically as a result of the action of several proteases (4, 7, 12). This conversion has been demonstrated with enzymes of animal origin, such as trypsin and ecarin, and more recently with bacterial enzymes, such as the thioprotease of *Bacteroides melaninogenicus* (15) and the neutral protease of *Pseudomonas aeruginosa* (10). The plasma-clotting effect produced by some strains of these two species of bacteria has often been erroneously reported to be the result of a coagulase action, whereas a true coagulase activity, as defined by the formation of an active coagulase-prothrombin complex, has only been demonstrated in *Staphylococcus aureus* (staphylothrombin; 16) and *Peptococcus indolicus* (peptothrombin; 13).

In *S. aureus*, coagulase activity is commonly regarded as being directly related to the pathogenicity of the organism, and therefore its activity is routinely tested in clinical laboratories. However, we have observed that some strains of *S. aureus* will not coagulate plasma, if protease inhibitors are present, although the normal coagulase activity is unaffected by the presence of these inhibitors (14).

In this communication we demonstrate that this unusual activation of prothrombin in *S. aureus* might be due to the action of metalloprotease.

MATERIALS AND METHODS

S. aureus metalloprotease and protease were isolated and purified as described elsewhere (3, 5). The enzymes were added in the amount of 500 μg to a solution of purified bovine prothrombin [40 IU in tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer, 0.15 M, pH 7.0] prepared according to Malhotra and Carter (8). Final volume of the mixture was 1 ml. Incubation was carried out at 37°C. Samples of 50-μl volume were taken at different time intervals and tested for thrombin activity by the estimation of the clotting time after addition of 200 μl of a 0.25% solution of bovine fibrinogen (6) in the Tris-hydrochloride buffer, and by determination of its activity on the synthetic substrate Chromozym TH (Boehringer, Mannheim, West Germany). The substrate in an amount of 0.2 mmol was prepared in Tris-hydrochloride buffer, 0.15 M (pH 7.6), and the activity was determined by measuring the optical density at 405 nm with a Beckman 25 kinetic model spectrophotometer.

In the controls, thrombin formation from prothrombin solution was measured after addition of 5 μg of ecarin, a venom from *Echis carinatus* (Pentapharm). The substrate solution was used.

RESULTS

The metalloprotease from *S. aureus* can generate thrombin activity upon incubation with prothrombin, whether it is measured by the appearance of clotting of pure fibrinogen or the synthetic substrate Chromozym TH (Fig. 1). The degree of prothrombin activation by the metalloprotease was similar to that caused by ecarin, tested as a control, although ecarin promoted thrombin activity much earlier. Addition of ethylenediaminetetraacetic acid, an inhibitor of the metalloenzyme, at a concentration of 0.4 mmol/ml resulted in a complete inhibition of prothrombin activation by the metalloprotease as determined by either one of the two assay procedures mentioned above. That ethylenediaminetetraacetic acid had no direct effect on prothrombin was revealed by the appearance of thrombin activity upon the addition of 5 μg of ecarin per ml (7) to the inhibited reaction mix-

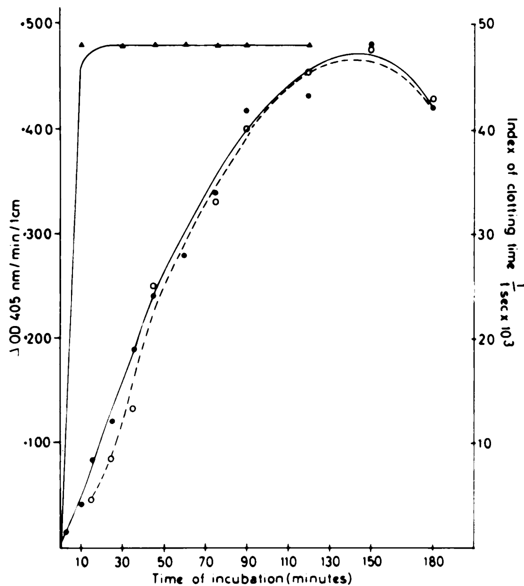

FIG. 1. *Thrombin formation during incubation of prothrombin with staphylococcal metalloprotease. Symbols:* (○) *clotting time after addition of fibrinogen,* (●) *digestion of the synthetic substrate (Chromozym TH),* (▲) *control after addition of ecarin.*

ture. Staphylococcal protease, another proteolytic enzyme from *S. aureus* (5), but known to have substrate specificity different from that of the metalloprotease, did not activate prothrombin.

DISCUSSION

Activation of prothrombin by the staphylococcal neutral protease has been demonstrated. Since *S. aureus* and *Staphylococcus epidermidis* strains are both producers of the metalloprotease (1, 2, 11), it is tempting to postulate that the disseminated intravascular clotting effect observed after or during systemic infection by staphylococci might be the result of the action of the metalloprotease, in addition to that of the coagulase. These observations might also help to explain why some coagulase-negative strains of *S. epidermidis* are able to cause septicemia (9). The presence of the metalloprotease in cultures of staphylococci might, therefore, influence coagulase activity testing. The presence of ethylenediaminetetraacetic acid in the reaction mixture was therefore recommended to eliminate the possibility of an activity resulting from the action of the metalloprotease.

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