Comparison of the Affinities to Bovine and Human Prothrombin of the Staphylocoagulases from *Staphylococcus intermedius* and *Staphylococcus aureus* of Animal Origin

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Staphylocoagulases of *Staphylococcus aureus* (40 strains originally isolated from horses, dogs, cats, cows, sheep, opossums, pigs, humans, and a goat) and *Staphylococcus intermedius* (19 isolates from dogs and 1 pigeon strain) were tested for their affinity to prothrombins of either bovine or human origin. The tests used were the coagulate tube test (using human, bovine, or equine fibrinogen with either bovine or human prothrombin as the source of coagulase-reacting factor) and a chromogenic assay which enabled quantification of the amidolytic activity of the staphylocoagulase-prothrombin complex. *S. intermedius* showed weak specificity for human prothrombin, with 15% of the coagulases clotting human fibrinogen, 25% clotting equine fibrinogen, and 40% clotting bovine fibrinogen. However, 65% of coagulases clotted equine fibrinogen, 75% of coagulases clotted human fibrinogen, and 100% of coagulases clotted bovine fibrinogen when bovine prothrombin was used. The animal isolates of *S. aureus* displayed more diverse specificity toward prothrombin than *S. intermedius* strains. While 85% of coagulate preparations clotted human fibrinogen when human prothrombin was used, only 45% of preparations clotted bovine fibrinogen when bovine prothrombin was used. However, 62.5% of coagulases clotted human fibrinogen when bovine prothrombin was used and 85% of coagulases clotted bovine fibrinogen when human prothrombin was used. This may be a reflection of the diversity of the animal origins of *S. aureus* isolates.

Staphylocoagulase is the extracellular protein produced by some *Staphylococcus* species which forms a stoichiometric complex with plasma prothrombin (8, 9). The formation of this staphylocoagulase-prothrombin complex usually leads to the exposure of the active site on the prothrombin molecule. Thus, this complex is capable of splitting fibrinogen into fibrin, and fibrin then forms a clot in a manner similar to that of biologically activated prothrombin (viz., thrombin) (19, 22). The differences in the abilities of various *Staphylococcus* species to clot plasma samples from different animals have been recognized for many years (3). For example, some strains of *Staphylococcus aureus* isolated from humans were able to clot, within 3 h of incubation, either bovine or sheep plasma as well as human plasma, although other *S. aureus* strains could not (7, 13, 17). In contrast, *Staphylococcus intermedius* isolates from dogs failed to clot human plasma while clotting of bovine or canine plasma occurred (6, 12, 17). To overcome the possibility of false coagulase results, some researchers have suggested using plasma from the animal species from which the organism had been isolated (13, 17). However, as most of the diagnostic work at the time was concerned with human isolates, the use of rabbit plasma proved satisfactory. Initial research into the mechanism of coagulation with staphylocoagulase dealt with human *Staphylococcus* strains. These staphylocoagulases often failed to clot plasma samples from cows and various other animals (2, 19, 22). This failure was attributed to the lack of coagulase-reacting factor (2, 14, 19). Coagulase-reacting factor was later shown to be either prothrombin or a part of it, prethrombin 1 (8, 9). Alternate explanations for this failure, such as an affinity for certain plasma samples or the presence of different coagulase-reacting factors from different animal species, have been suggested (1, 21). Hendrix et al. (9) showed that the staphylocoagulase from *S. aureus* exhibited highly species-dependent specificity toward human prothrombin but showed none toward bovine prothrombin. Engels (4), on the basis of what was known about the mode of action of staphylocoagulase, suggested that the limiting step in clotting plasma samples of different animal origins could be the specificity of the staphylocoagulase toward certain species’ prothrombin molecules. This latter theory seems to be more likely than the alternate possibility of species specificity toward the fibrinogen molecule. In this paper we have tried to determine whether strains of *S. intermedius* showed any difference in their affinity toward prothrombins of different animal origins. Because many strains of *S. intermedius* failed to clot human plasma, prothrombin of human origin was chosen for comparison with bovine prothrombin, as bovine plasma undergoes coagulation with *S. intermedius* strains. Staphylocoagulases from strains of *S. aureus* were used for comparison with *S. intermedius* staphylocoagulases to investigate a possible specificity which may influence the outcome of coagulation of plasma samples from different sources.

**MATERIALS AND METHODS**

**Bacterial strains and culture conditions.** A total of 60 strains were used. Twenty strains of *S. intermedius* (19 isolates from dogs with clinical disease and 1 strain from a pigeon [CCM 5739; Czechoslovak Collection of Microorganisms]) and 40 strains of *S. aureus* (11 strains isolated from horses, 7 from dogs, 5 from cats, 4 each from cows and sheep, 3 from opossums, 2 from pigs, 1 from a goat, and 3 from humans [Cowan 1, one strain from Adelaide Hospital, and the Smith strain from the United States donated by Sydney Hospital]) were used.
The cultivation media and culture conditions were as described previously (16).

**Preparation of crude staphylocoagulase.** The method of Soulier et al. (16, 18) was used for the preparation of crude staphylocoagulase.

**Preparation of fibrinogen.** Human fibrinogen was received from C.S.L. (Melbourne, Australia). Bovine blood samples were collected during slaughter directly into 3-liter flasks containing 3.8% (wt/vol) trisodium citrate as an anticoagulant (10% [vol/vol]). Horse and sheep blood samples were collected into commercial packs (Tuta Primary Pack-Citrated Phosphate Dextrose [formula 050]; Tuta Laboratory, Lane Cove, N.S.W., Australia). Fibrinogen was prepared by the Blomback modification of Cohn’s ethanol fractionation (11, 15), yielding 85% clottable protein. The stock solution was stored in 10-ml aliquots in liquid nitrogen. To minimize the effect of residual prothrombin, the stock fibrinogen solution was mixed with barium sulfate (1% [wt/vol]) and gently stirred for 10 min at room temperature and the precipitate was removed by centrifugation at 2,000 x g for 10 min. The supernatant solution was then immediately used in the coagulase tube test.

**Coagulase tube test.** One hundred microliters of crude preparation of staphylocoagulase and 10 µl of either human or bovine prothrombin solution or saline were added to 300 µl of the stock fibrinogen solution. The tubes were incubated for 3 h at 37°C. A solid clot was taken as a positive result.

**Staphylocoagulase testing.** The chromogenic method of Engels et al. (5) was used with either human (Prothrombinex; C.S.L., Melbourne, Australia) or bovine (F4253; Sigma Chemical Co., St. Louis, Mo.) prothrombin as the source of coagulase-reacting factor. These prothrombins were also used for the coagulase tube test. The method used was essentially as described previously (16), although the concentration of prothrombin was reduced by a factor of 100 without appreciable effect on the result. Also, the assay time was shortened to 30 min to accommodate rapid hydrolysis of some staphylocoagulase preparations.

**RESULTS AND DISCUSSION**

Coagulation of plasma samples containing anticoagulants such as citrate, oxalate, heparin, EDTA, etc., by some *Staphylococcus* species is caused by the extracellular protein, staphylocoagulase. In order to test species specificity, the absorbed stock fibrinogen was used with or without the addition of either human or bovine prothrombin. This would absorb some of the residual prothrombin still present in the stock fibrinogen solution and minimized its effect on the clotting process. The results of the coagulase tube test are presented in Table 1. From these results it seems that *S. intermedius* showed a very weak specificity for human prothrombin, which was reflected by a failure of 85% of the staphylocoagulase preparations to clot human fibrinogen. On the other hand, when bovine prothrombin was used, 75% of the staphylocoagulase preparations formed a clot with human fibrinogen and 100% of the staphylocoagulase preparations formed a clot with bovine fibrinogen. Small traces of residual prothrombin in the fibrinogen solution might have caused somewhat higher positive results with bovine fibrinogen for *S. intermedius*. When human prothrombin was used, 15% of the coagulases clotted human fibrinogen, 25% of the coagulases clotted horse fibrinogen, and 40% clotted bovine fibrinogen. *S. aureus* strains differed in their specificity, with only 15% of coagulase preparations failing to clot human fibrinogen when human prothrombin was used. However, some 55% of *S. aureus* staphylocoagulase preparations failed to clot bovine fibrinogen when bovine prothrombin was used. In contrast with most other *S. aureus* strains, staphylocoagulases from two pigs, two cows, one cat, and one sheep failed to clot human or equine fibrinogen when human prothrombin was used. Thus, the origin of the fibrinogen did not appear to be the determinant factor in the coagulation process tested.

The animal isolates of *S. aureus* displayed more diverse specificity toward prothrombin than *S. intermedius* strains. Figure 1 shows the marked increase in hydrolyzation of the chromogenic substrate (Chromozym-TH) by *S. intermedius* strains when bovine prothrombin instead of human prothrombin was used. While the majority of *S. aureus* species responded to human prothrombin with increased hydrolyzation of Chromozym-TH, some 20% of strains did not show much difference in affinity to either prothrombin and some 7.5% of other strains had a reverse specificity to the majority of *S. aureus* strains. Unfortunately, the small number of isolates from different animal hosts did not enable a conclu-

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<th>Table 1. Test tube coagulation of animal staphylocoagulases</th>
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<td><strong>Staphylococcus isolates</strong> (a)</td>
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<td><strong>S. intermedius</strong> (20)</td>
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*Three different stock fibrinogens (300 µl) were used (human, bovine, and equine). Columns: a, no additional prothrombin added; b, 10 µl of human prothrombin added; c, 10 µl of bovine prothrombin added. A 100-µl sample of crude staphylocoagulase preparation was added and incubated at 37°C for 3 h. Only a solid clot was taken as a positive result.*
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FIG. 1. Hydrolyzation of chromogenic substrate (Chromozym-TH) in 30 min by staphylocoagulase preparation (16) of the 60 Staphylococcus isolates. Human (△, ■) and bovine (Δ, ○) prothrombin were used as the source of coagulase reaction factor. Symbols: Δ and △, S. intermedius (isolate 1 to 20); ■ and ○, S. aureus (isolates 21 to 60).

sion to be drawn about the affinity of the individual host species staphylocoagulases to prothrombin. In general, the results obtained with the chromogenic assay were in agreement with the results from the coagulase tube test.

From purification studies, estimates of molecular weights (22), studies on immunogenic responses for different staphylocoagulases (10, 20), as well as the variation in specificity to different prothrombins reported here, it appears that the extracellular proteins collectively named staphylocoagulase are quite heterogeneous.

ACKNOWLEDGMENT

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REFERENCES