Protein A in *Staphylococcus aureus* Isolates from Pigs

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ABSTRACT. The presence and quantity of protein A in *Staphylococcus aureus* 147 isolates from the tonsils of healthy pigs were examined by three methods. Cell-bound protein A was detected in 71 (48%), 104 (71%) and 123 (84%) of 147 isolates by the slide hemagglutination test, microplate hemagglutination test and enzyme-linked immunosorbent assay (ELISA), respectively. Extracellular protein A was not detected in any isolates by the microplate hemagglutination test. When the quantity of cell-bound protein A in the isolates was determined by the ELISA, most of the isolates contained about 0.8 to 2.2 μg of protein A/m*l* in bacterial cell suspensions of a concentration of MacFarland No. 3.—KEY WORDS: protein A, *Staphylococcus aureus*, swine.

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Protein A from *Staphylococcus aureus* is covalently linked to the peptidoglycan structure of the cell wall [3]. However, in some methicillin resistant strains of *S. aureus*, it may occur as an extracellular secretion without passing through a cell wall-bound state [3]. Protein A possesses the capability of binding the Fc region of immunoglobulins from various mammalian species [4, 8]. Therefore, many researchers have been using it as a useful tool in immunoglobulin-associated experiments. In addition, protein A activates serum complement, induces chemotaxis and hypersensitivity reactions and inhibits phagocytosis [3, 6]. From the biologic effects, protein A seems to be an important virulence factor in the genesis of staphylococcal infections.

The presence of protein A has been demonstrated in most isolates of *S. aureus* from human [2, 5] and in many isolates from cattle [7, 9, 12]. Moreover, it has been detected in *Staphylococcus intermedius* isolates from dogs and cats [1, 17] and in *Staphylococcus hyicus* subsp. *hyicus* isolates from pigs [10, 11, 15]. But there is little information concerning the quantity of protein A among *S. aureus* isolates from pigs. The present study was thus conducted to examine the presence and quantity of cell-bound and extracellular protein A in *S. aureus* isolates from pigs by a slide hemagglutination test, microplate hemagglutination test and enzyme-linked immunosorbent assay.

A total of 147 swine isolates of *S. aureus*, described in the previous study [16], were examined in the present experiments. These isolates were obtained from the tonsils of about 80 healthy pigs killed at a slaughter house in Fukui prefecture. *S. aureus* strain Cowan I and strain Wood 46 were used as protein A positive and negative controls, respectively.

Cell-bound protein A in the isolates was examined by the slide hemagglutination test, described by Winblad and Ericson [18]. In the test, 3% sensitized sheep red cell solution, which was prepared with rabbit antiserum for sheep erythrocytes, was used. As a result, cell-bound protein A was detected in 71 (48%) of 147 isolates, when the appearance of hemagglutination within 5 min was judged to be positive for protein A. In particular, 55 (37%) isolates showed strong hemagglutinating reaction within 1 min, likewise to that of *S. aureus* strain Cowan I. *S. aureus* strain Wood 46, used as negative control, did not show any hemagglutinating reaction in the test. The percentage of protein A-positive in swine isolates of *S. aureus* was low as compared to those of human isolates (90%) and bovine isolates (69 to 77%) described by some workers [5–7, 12].

Next, cell-bound and extracellular protein A in the isolates were determined by the microplate hemagglutination test described previously [15]. Briefly, the isolates were cultured into heart infusion broth at 37°C for 18 hr and centrifuged. The resulting culture supernatants were used for extracellular protein A. The bacterial cells in sediments were digested by lysostaphin (Sigma), according to the method described by Sjöquist *et al.* [13]. After centrifugation, the supernatants from digested bacterial cells were used for cell-bound protein A. Twenty-five μl of the supernatants from digested bacterial cells or culture supernatants was serially diluted twofold with PBS containing 0.1% bovine serum albumin (Sigma) in microtiter plates. An equal volume of 1% sensitized sheep red cells was added to each well of the plates. Then the plates were shaken and incubated at 37°C for 2 hr.

As a result, cell-bound protein A was demonstrated in 104 (71%) of 147 isolates when above 16 of hemagglutinating titer was judged as positive. The result indicates that the microplate hemagglutination test is highly sensitive than the slide hemagglutination test. As shown in Fig. 1, the hemagglutinating titers of most isolates, showed positive in the slide hemagglutination test, ranged from 32 to 512. But the hemagglutinating titers of 12 isolates were much lower from <2 to 8. These isolates showed strong proteolytic ability when were cultivated on a skim milk agar plate. From the result, it is conjectured that protein A of 12 isolates may have been hydrolyzed by their proteolytic enzymes in the process of digestion of bacterial cells with lysostaphin. The titers of negative isolates in the slide hemagglutination test ranged from <2 to 128, but most titers were less than 64. In the previous study [15], we conjectured that the hemagglutinating reaction of low titers (2 to 8) is not due to protein A. So it seems that the low titers in the present experiment may be also nonspecific hemagglutinating reaction. On the other hand, extracellular protein A was not detected in any isolates when examined by using the culture supernatants. Forsgren *et al.* [3] described that some methicillin resistant isolates of *S. aureus* produces exceptionally high concen-

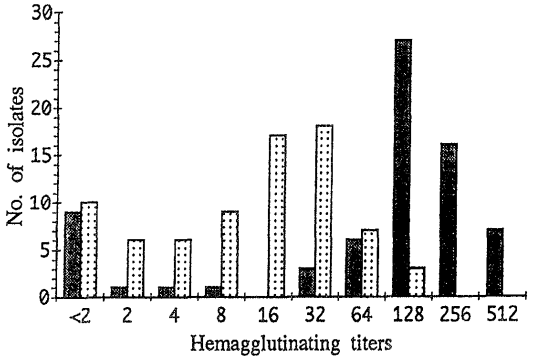

Fig. 1. Quantitative determination of cell-bound protein A in swine isolates of *S. aureus* by the microplate hemagglutination test. ▩: Positive. ⊡: Negtive by the slide hemagglutination test.

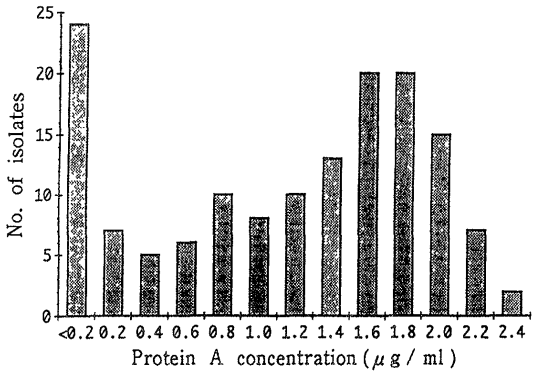

Fig. 2. Distribution of concentrations of cell-bound protein A in bacterial cell suspensions of MacFarland No. 3 of swine isolates, which was determined by the ELISA.

trations of extracellular protein A. But all of the isolates used in the present experiments were methicillin sensitive as described previously [16].

The quantity of cell-bound protein A in isolates was examined by the enzyme-linked immunosorbent assay (ELISA) described previously [14]. Briefly, 100 μl of the bacterial cell suspensions, diluted 20-fold of a concentration of MacFarland No. 3, was coated to wells in microtiter plates and incubated at 37°C for 16 hr. After washing, 100 μl of peroxidase-conjugated rabbit anti-swine IgG (Organo Teknika Corporation), diluted at 1:1,000, was added to each well of the plates and incubated at room temperature for 1 hr. After washing 100 μl of substrate solution (2,2-azino-di-[3-ethyl-benzthiazoline-sulfonate]) was added to each well. After 30 min incubation at room temperature, the reaction was stopped and the optical density (OD) was measured. Recombinant protein A (Repligen Corporation, Massachusetts) was used as a standard sample in the ELISA.

In the ELISA, OD values of >0.201 were considered to be positive for protein A because the OD value of *S. aureus* strain Wood 46 was <0.150. As a result, cell-bound protein A was demonstrated in 123 (84%) of 147 isolates. The OD values of most isolates ranged from 0.4 to 2.5. There was a correlation between the OD values of ELISA and hemagglutinating titers in many isolates. The OD values of isolates, showed hemagglutinating titers of >28, were >1.8.

When purified recombinant protein A was examined by ELISA, a linear relationship was demonstrated between the OD values and the concentrations of 20 to 80 ng of protein A/m*l*. Therefore, the quantity of cell-bound protein A in isolates was estimated by reference to this standard curve. As shown in Fig. 2, most of the isolates contained about 0.8 to 2.2 μg of cell-bound protein A/m*l* in bacterial cell suspensions of a concentration of MacFarland No. 3. In particular, 44 (30%) isolates produced >1.8 μg of cell-bound protein A/m*l*. Strain Cowan I of *S. aureus*, used as positive control, produced >1.8 μg of cell-bound protein A/m*l*. From the results, it is conjectured that about 30% of swine isolates may have been produced as much cell-bound protein A as did strain Cowan I originating from human.

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