Iron-Dependent Synthesis of Hemolysins by *Staphylococcus aureus*

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We studied the effect of Fe^{2+} on hemolysin synthesis by *Staphylococcus aureus*. Hemolytic activity of staphylococci was shown to be iron-dependent. Addition of Fe^{2+} to the nutrient medium induced the synthesis of α-hemolysin by staphylococci. Increasing the concentration of Fe^{2+} was followed by an increase in hemolytic activity of staphylococci. Inducible synthesis of α-hemolysin probably serves as an important pathogenetic factor in the development of staphylococcal infection in patients with excessive iron accumulation in the body.

Key Words: *iron; Staphylococcus aureus; hemolytic activity; hemolysins*

Despite intensive studies of biochemical properties of *Staphylococcus aureus* (*S. aureus*), this bacterium remains the major pathogenetic agent of inflammatory purulent and septic processes. Hemolysin production by staphylococci is an important factor of their pathogenicity. Hemolytic activity provides bacteria with an additional source of iron (hemoglobin from destroyed erythrocytes) [1]. Surface proteins of *S. aureus* capture hemoglobin, release heme from hemoglobin, and transport it across the cell wall and plasma membrane into the cytoplasm [8]. Previous studies showed that hemolysin synthesis in *Vibrio cholerae* increases under conditions of iron deficiency, but is inhibited by excess iron in the nutrient medium [4]. However, the effect of iron in various concentrations on hemolysin synthesis by *S. aureus* remains unknown. These studies will elucidate the specificity of hemolysin expression by *S. aureus* under various conditions.

Here we evaluated the effect of Fe^{2+} in various concentrations on hemolysin synthesis by *S. aureus*.

MATERIALS AND METHODS

Experiments were performed on standard strain *S. aureus* ATCC 25,923 and 2 isolates from the blood (372) and wound exudate (4844) of patients at the Khanty-Mansiysk regional clinical hospital. Baseline hemolytic activity (HA) was measured photoelectrocolorimetrically by lysis of human erythrocytes (0(I), Rh(+)) by bacterial supernatants [2]. The solutions and nutrient media were prepared on deionized water. The effect of Fe^{2+} on HA of bacteria was studied using iron-deficient nutrient medium (IDM) in meat-peptone broth (scientific production association "Pitatel'nye sredy") [5,6]. Iron concentration in the original IDM was taken as zero. The study was conducted with Fe^{2+} in concentrations of 0, 4.0 (optimal for growth), and 50.0 μM (excess). Fe^{2+} was added to the nutrient medium in the form of iron sulfate (II).

Bacteria were grown a meat-peptone agar slant at 37°C for 12 h and washed with physiological saline. The microbial suspension was titrated to an optical density of 0.500-0.510 optical units.

Iron sulfate (II) in the specified concentration, 0.5 ml 5% human erythrocyte suspension (0(I), Rh(+)) in physiological saline, and 0.1 ml microbial suspension were added to 2 ml sterile IDM. In the control, sterile physiological saline (0.1 ml) was added instead of microbial suspension. The inocula were cultured at 37°C for 0, 3, 6, 9, 12, and 24 h and centrifuged at 3000*g* for 15 min. Optical density of the supernatant was measured against water (λ=543 nm). HA was expressed in percentage and calculated as follows:

$$HA=((D_o-(D_{k1}+D_{k2}))/D_n)\times 100,$$

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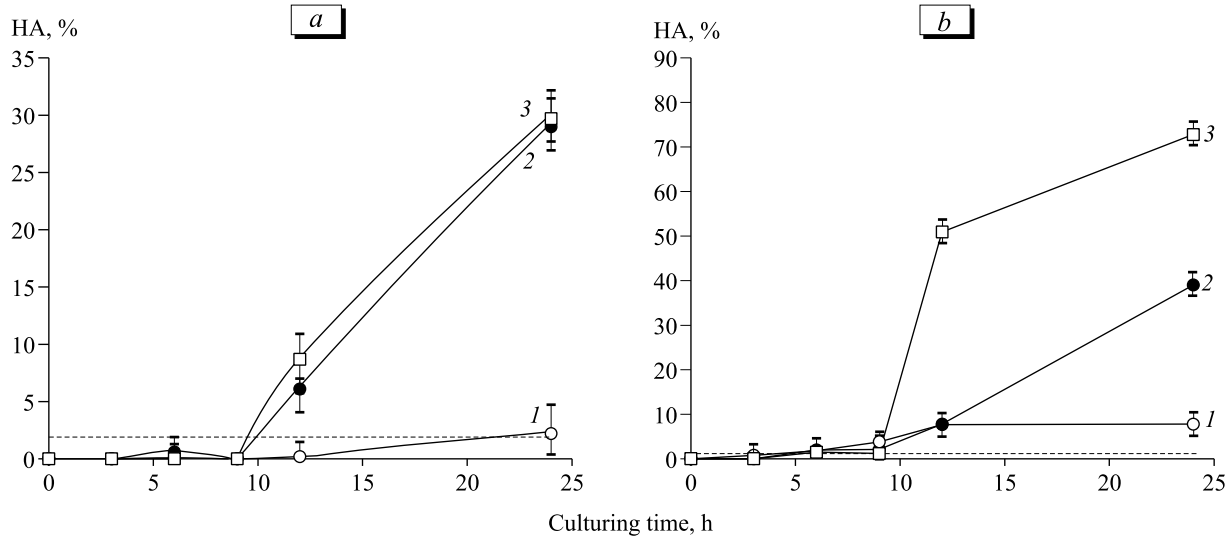

Fig. 1. Hemolysis curves for *S. aureus* 25,923 (*a*) and 4844 (*b*) at various concentrations of Fe^{2+} in the nutrient medium. Dotted line: baseline HA; HA in the medium without Fe^{2+} (*1*); HA in the presence of Fe^{2+} at concentrations of 4 (*2*) and 50 μM (*3*).

where D_o is optical density of treated samples; D_{k1} is optical density in the control for meat-peptone broth (mixture of 2 ml broth, 0.5 ml 5% erythrocyte suspension, and 0.2 ml physiological saline); D_{k2} is optical density in the control for 5% erythrocyte suspension (mixture of 2.2 ml physiological saline and 0.5 ml 5% erythrocyte suspension); and D_n is optical density of a completely hemolyzed sample (mixture of 0.5 ml 5% erythrocyte suspension, 2.2 ml physiological saline, and 2 mg saponin).

The results were analyzed by Student's *t* test.

RESULTS

Baseline HA of staphylococci was 1.8-2.2%. This low level of HA is usually considered as insignificant and is interpreted as the absence of activity. The test strains produced no hemolytic zones after 12-h culturing on blood agar.

Figure 1 shows hemolysis curves for 2 strains of *S. aureus* at various concentrations of Fe^{2+}. Staphylococcal hemolysis in iron-containing medium was described by *S*-shape curves, which is typical of α-hemolysin [7]. HA of ATCC strain 25,923 and isolate 4844 remained practically unchanged in the absence of Fe^{2+} in the nutrient medium (0.2-2.2 and 0.8-7.8%, respectively). Addition of Fe^{2+} to a concentration of 4 μM in the nutrient medium was followed by a significant increase in HA (compared to the control). HA of ATCC 25,923 and isolate 4844 was maximum after 24-h culturing (29 and 39%, respectively). HA increased more significantly in the presence of Fe^{2+} in a concentration of 50 μM (as compared to 4 μM Fe^{2+}). HA of ATCC 25,923 and isolate 4844 was highest after 24-h culturing under these conditions and reached 29.7 and 72.8%, respectively. Fe^{2+} was shown to induce HA of *S. aureus* isolate 372 (from the blood), ATCC 25,923, and isolate 4844. HA was highest after 24-h culturing with 50 μM Fe^{2+} (21.9%).

Comparison of the results showed that standard strain *S. aureus* ATCC 25,923 and isolates from the blood and wound exudate did not differ by HA at Fe^{2+} concentration of 4 μM. Increasing the concentration of Fe^{2+} to 50 μM had little effect on HA of the standard strain *S. aureus* ATCC 25,923, but significantly increased this parameter for isolates 372 and 4844.

Our results indicate that addition of Fe^{2+} to the nutrient medium induces production of α-hemolysin by staphylococci, which were initially classified to non-hemolytic agents. These data explain the specificity of hemolysin expression under various conditions. Previous observations showed that humans with abnormally high accumulation of iron are predisposed to infectious diseases induced by attenuated pathogens [3]. Inducible synthesis of α-hemolysin by staphylococci serves as an important pathogenetic factor in the development of staphylococcal infection.

Therefore, α-hemolysin synthesis by *S. aureus* is iron-dependent. These results can be used to evaluate the role of hemolysins in biological activity of staphylococci. Probably, staphylococcal α-hemolysin is an iron-containing protein. Hence, α-hemolysin synthesis is stimulated by bivalent iron ions.

We conclude that the use of blood agar with exogenous iron (not less than 4 μM) or preparation of the nutrient medium with tap water (preliminary estimation of Fe^{2+} concentration) holds much promise for the standard measurement of *S. aureus* HA.

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