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Study on factor H mediated identification of staphylococcal Sbi protein in pure culture

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ABSTRACT

Staphylococcal Sbi is an IgG binding protein having high affinity for Fc region of human IgG similar to SpA. This bacterial pathogen masks itself with host regulator Factor H and inhibits phagocytosis and opsonization. Factor H has been found to be present in normal human serum (NHS). Thus identification of Factor H facilitates detection of Sbi protein when staphylococcal cells were incubated with NHS. In our present work, we studied the identification of Staphylococcal Sbi protein: Factor H complex using Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot analyses with polyclonal anti-Factor H antibodies.

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KEYWORDS

Staphylococcus aureus;
Sbi;
Factor H;
SDS-PAGE;
Western blot.

INTRODUCTION

Staphylococcus aureus (*S. aureus*), the most prevalent pathogen of humans, cause up to one third of all bacterial diseases ranging from boils and pimples to food poisoning, to septicemia and toxic shock. *S. aureus* is a gram positive bacterium that permanently colonizes the human skin and mucous membranes of approximately 20% of the population[1]. Once the pathogen crosses host immune barriers, *S. aureus* can cause superficial skin infection, toxin mediated diseases [2] or serious invasive infections depending on the interaction of the pathogen's virulence factors and the defense mechanisms of the host[3]. The ability of *S. aureus* to cause infections is partly due to proteins that are anchored to the cell surface and to those that are secreted into the medium like hydrolytic enzymes, toxins and proteins with immune evasion functions[4]. Sbi is one such staphylococcal IgG binding protein having high affinity for Fc region of human IgG similar to SpA[5]. IgG binding of Sbi was confirmed for one polyclonal antiserum and two monoclonal antibodies (mABs), which are directed to factor H[6].

Short Communication

Factor H (FH) is a single – chain serum glycoprotein of 150 KDa with a modular structure consisting of a tandem of 20 homologous units of about 60 amino acids, called SCR (short concensus repeat) and is present at 500 mg/ml in blood plasma[7]. In order to restrict complement activation to the surface of an invading microbe host cells are protected from complement attack by membrane bound and soluble regulators[6]. Bound to the surface of the pathogen; these host regulators retain complement regulatory functions and inhibit complement activation. Therefore, acquisition of host regulators masks the pathogenic surface which results in survival of the pathogen[8,9].

Therefore, in our present work, we studied the formation of Factor H:Sbi complex towards facilitating the subsequent study of pathogenicity of *Staphylococcus aureus* NCIM 2602 in pure culture and food samples.

MATERIALS AND METHODS

Bacterial strain and culture condition

Staphylococcus aureus NCIM 2602 was procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune. *S. aureus* was grown at 37^0C for overnight in nutrient broth (Beef Extract-10.0g, NaCl-5.0g, Peptone-10.0g, Agar-20.0 per liter distilled water).

Serum sample collection

Normal human serum (NHS) was procured by collection of blood sample in vacuatte tube. The tube was kept for 2 hours at 37^0C. The serum layer comes above and the red blood cells settle at the bottom. The serum was obtained from the top and washed at 3000 rpm for 5 min so that the remaining red blood cells accumulate at the bottom and the pure serum was obtained from the surface. The pure serum sample was prepared in sample buffer for SDS-PAGE analysis, according to the procedure[10] and presence of Factor H was determined comparing with prestained molecular weight marker (Fermentas).

Isolation of Sbi protein from *S. aureus* and Identification of Sbi bound FactorH complex

Cells were harvested by centrifugation (Model - Hanil 22K) at 9700 rpm for 8 min. Cells were further resuspended in veronal buffered saline supplemented with 10 mM EDTA and incubated with normal human serum (NHS) for 2 hours at 37^0C with agitation. After incubation cells were washed four times with EDTA- veronal buffered saline and bound proteins were eluted with SDS-buffer (60 mM Tris-HCl,pH – 6.8, 2% SDS, 25% glycerine) for 5 min at 98^0C and analysed bySDS-PAGE and Western Blot.

Antigen-antibody binding

The proteins were electrotransferred to polyvinylidinedifluoride (PVDF) membrane for Western blotting using anti-Factor H polyclonal antibody (pAb) (Calbiochem) developed in goat as primary antibody and horseradish peroxidase (hrp) coupled anti-goat developed in rabbit IgG as secondary antibody for detection of Sbi bound factor H complex. The membrane was incubated with anti-factor H goat pAb supplemented with 5% skimmed milkat 1:1000 µlto avoid non specific binding between antigen and antibody. Diaminobenzinidine (DAB) and H_2O_2were togetherlyused as substrate for hrp labeled secondary antibody[11].

RESULTS AND DISCUSSION

The molecular weight of Factor H protein was found nearly around 150 KDa (Figure 1). Sbi could not be detected alone as it is a cell secreted protein. Therefore to identify and locate Sbi protein, Staphylococcal cells were incubated with NHS containing Factor H component. Sbi has strong affinity for Factor H to form Sbi: Factor H complex[6]. SDS-PAGE analysis of the final reaction solution containing *S. aureus* NCIM 2602 cells and NHS showed the presence of a band between 170 and 130 KDa. Therefore we could assume that the band not only representedFactor H alone but Sbi protein was also associated with it (Figure 2). Like many other human pathogens, *Staphylococcus aureus*, a gram positive bacterium binds the complement regulator Factor H from human serum. Staphylococcal Sbi protein acts as ligand for such complement regulator[5]. After the transfer of the protein complex from gel to the membrane,

further confirmation was made using anti-Factor H polyclonal Antibody (pAb) and hrp conjugated secondary antibody. DAB was used as the substrate for color reaction with hrp conjugated secondary antibody. Just before use, 0.02% H_2O_2 was mixed with DAB and the membrane was incubated for 5 to 15 min. Electrons are transferred by hrp from DAB to the peroxide to yield an insoluble brown product (Figure 3). Sbi protein masks itself by binding with Factor H and thereby protects itself from phagocytosis and opsonization. Thus Staphylococcal Sbi protein very effectively interferes with human immune system and causes major health related problems. As because *S. aureus* contamination easily occurs

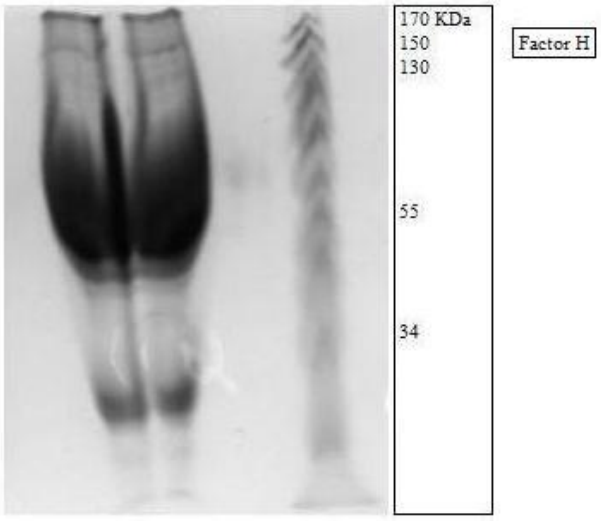

Figure 1 : SDS-PAGE analysis of Factor H in normal human serum.

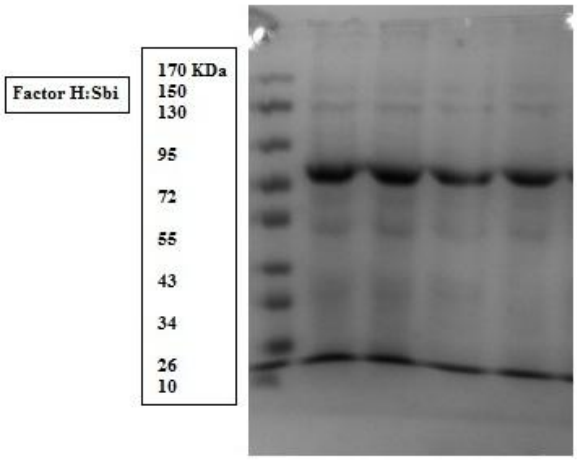

Figure 2 : SDS-PAGE analysis of Sbi: Factor H complex at 150 KDa.

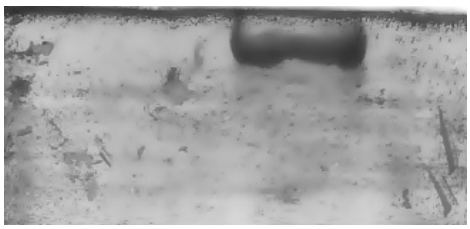

Figure 3 : Western blot analysis for identification of Sbi-Factor H complex using anti-Factor H pAb developed in goat as primary antibody and horse radish peroxidase (hrp) conjugated anti-goat developed in rabbit IgG as secondary antibody.

in human beings through regular food constituents (milk, meat, cheese etc) consumption, detection of such pathogen has been proved to be a very vital aspect in the field of medical microbiology. This work has been done with a view to extend in a future study of the formation of the complex, Sbi: Factor H in food samples contaminated with *S. aureus*.

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