Short Research Article

Identification of Hemin binding protein of oral streptococci via electrophoresis in SDS polyacrylamide gel

ABSTRACT

Background and Objectives: It has been reported that hemin binding proteins are involved in the mechanism of obtaining iron in some bacteria. Oral streptococci in the dental plaque are assumed to acquire iron through hemin or hemin compounds. The purpose of this study was to identify the presence of a protein (hemin binding protein) involved in the hemin binding mechanism of oral streptococci.

Methodology: In this study, we investigated the presence of proteins involved in hemin binding of oral streptococci through sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) analysis using hemin-agarose beads.

Results: As a result of SDS-PAGE analysis, similar or different sizes of hemin binding protein bands were observed depending on the strains belonging to streptococci. The molecular weight of hemin binding protein in *Streptococcus gordonii*, *Streptococcus rattus*, *Streptococcus sobrinus*, *Streptococcus sanguis* and *Streptococcus oralis* were approximately 95 kDa, 43 kDa, 43 kDa, 39 kDa, and 39 kDa, respectively.

Conclusion: In this study, the presence of hemin binding protein in streptococci was confirmed and the proteins involved in hemin binding in different species of oral streptococci may be different.

Keywords: Streptococcus, Electrophoresis, Hemin, Hemin binding protein

1. INTRODUCTION

Oral streptococci are gram-positive bacteria present in human oral cavity [1]. They are involved in the initial attachment stage of bacteria in plaque formation, and some are responsible for dental caries and bacterial infective endocarditis [1]. Iron is an essential nutrient for the growth of bacteria, which is also important for the metabolism of microorganisms. Iron is required for a variety of biochemical and physiological reactions, including oxygen binding proteins, heme and nonheme electron transport enzymes [2].

Currently, three mechanisms have been identified as the mechanism of iron accumulation of bacteria [3-5]. First, many aerobic and anaerobic bacteria produce low molecular weight iron-binding compounds called siderophores that are involved in iron transport [3]. After siderophore binds to iron, it binds to receptors present in bacteria. This allows bacteria to acquire iron [3]. Second, some bacteria have low-iron-inducible outer membrane proteins that can bind to human iron binding proteins such as transferrin or lactoferrin. They can obtain iron through iron binding proteins [4]. Finally, some bacteria have hemin binding proteins. They can obtain iron by binding heme or heme compounds such as hemoglobin

and myoglobin to heme binding proteins, and heme compounds can supply enough iron to grow bacteria [5].
However, streptococci does not have siderophores [6], and *Streptococcus mutans* does not utilize lactoferrin [7]. Therefore, streptococci are likely to obtain iron through heme and heme compounds. *Porphyromonas gingivalis*, as well as some other bacteria capable of acquiring iron through heme, have been associated with hemin binding proteins [8-10]. In this study, the presence of hemin binding protein in oral streptococci was confirmed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) using hemin-agarose beads [11].

2. MATERIAL AND METHODS

2.1 Bacteria and culture conditions

Streptococcus gordonii DL1 and *Streptococcus rattus* Fa-1f, *Streptococcus sobrinus* KN100, *Streptococcus sanguis* KN107, and *Streptococcus oralis* KN116 were used in the experiment. Streptococci were cultured in Todd-Hewitt Broth (Becton Dickinson biosciences, Franklin Lake, NJ, USA) for 18 hours at 37℃ in a CO_2 incubator containing 5% CO_2.

2.2 SDS-PAGE analysis using hemin agarose beads

SDS-PAGE analysis was performed to identify proteins involved in hemin binding of streptococci [11]. Streptococci were cultured in Todd-Hewitt liquid medium for 18 hours. The bacterial pellet obtained by centrifugation (12,000 x g, 4℃, 10 min) was washed twice with PBS. The bacterial pellet was suspended in 10 ml of Tris-NaCl buffer (TN buffer) (50 mM Tris + 1 M NaCl, pH 8.0) and left on ice for 10 minutes and crushed with an ultrasonicator (SONICS & MATERIALS, Newtown, Connecticut, USA) (left on ice for 30 seconds on and off 30 seconds). The suspension was centrifuged (12,000 x g, 4℃, 10 min) and 1 ml of the supernatant and 50 μl of hemin agarose beads (Sigma-Aldrich, Saint Louis, Missouri, USA) were incubated in a 37℃ water bath for 1 hour. When the reaction was complete, the supernatant was removed by centrifugation (7,000 x g, 4℃, 3 min). The resulting beads were suspended in 1 ml of Tris-NaCl buffer (pH 8.0) containing 10 mM EDTA and 0.75% sarcosyl. After the supernatant was removed by centrifugation, beads were suspended in 1 ml of 10 mM Tris-HCl (pH 8.0) to which 1 M NaCl, 10 mM EDTA and 0.5% sarcosyl were added, centrifuged again and the supernatant was removed (Repeat 3 times). The obtained beads were suspended in 1 ml of 50 mM Tris-HCl (pH 8.0) containing 1 M NaCl, then centrifuged (7,000 x g, 4℃, 3 min) and the supernatant was removed. (Repeat 2 times) 1 ml of TN buffer (pH 8.0) was added to suspend the beads and centrifuge (7,000 x g, 4℃, 3 min) to remove the supernatant. The beads were dissolved in 2X sample buffer and heated at 100℃ for 5 minutes. The 2X sample buffer was used for 1/2 of the bead volume. 20 μl of the prepared sample was electrophoresed on 10% polyacrylamide gel (KOMABIOTECH, Seoul, Korea). The gel was stained with coomassie brilliant blue (Sigma-Aldrich) [11].

3. RESULTS AND DISCUSSION

SDS-PAGE experiments showed different patterns of protein bands appearing to be involved in the hemin binding proteins of *S. gordonii* DL1 and *S. rattus* Fa-1f (Figure 1). The 95 kDa band of the predicted hemin binding-related protein observed in *S. gordonii* (Figure 1A, lane2) was not found in *S. rattus*. In *S. rattus*, a 43 kDa band was observed as an estimated hemin binding protein (Figure 1A, lane 3). The protein band of 43 kDa was not seen in *S.*

gordonii. A 43 kDa protein was observed as the hemin binding protein of *S. sobrinus* KN100 (Figure 1B, lane 2) and a 39 kDa protein was observed as the hemin binding protein of *S. sanguis* KN107 (Figure 1B, lane 3). In *S. oralis* KN116, a 39 kDa protein was observed as the hemin binding protein (Figure 1B, lane 4). Based on these results, it can be deduced that the proteins involved in hemin binding in different species of oral streptococci may be different from each other.

Previous studies have identified hemin binding proteins in some bacteria. In *Haemophilus influenzae*, a 39.5 kDa protein was founded as a protein involved in hemin binding [9]. In addition, in a study of *Vibrio vulnificus*, a 36.5 kDa hemin binding protein was observed to be involved in hemin binding [10]. And in the study on *P. gingivalis*, a 40 kDa protein was identified as a hemin binding protein [8]. The 40 kDa of *P. gingivalis* showed the ability to bind with hemin and was important for the growth of bacteria in hemin-poor environments [8]. These results suggest that the proteins involved in hemin binding may be different in each bacterium.

The SDS-PAGE analysis used in this study can infer the presence or absence of proteins involved in hemin binding, but did not provide any definitive information on the location of the hemin binding protein in bacteria.

Recently, Gao et al. [12] found a 18 kDa Dps protein homologue capable of binding to the hemin in *P. gingivalis*. In addition, Yamamoto et al. [13] reported a 20 kDa protein of the Dpr family, which is structurally similar to the Dps protein in *S. mutans*. This protein could bind to iron. Mieno et al. [14] showed that the Dpr protein of *S. mutans* and the Dps protein can develop into a heme-binding family (such as ferritin). In this study, 95 kDa, 43 kDa, 43 kDa, 39 kDa and 39 kDa proteins were identified as the possible hemin binding proteins of *S. gordonii, S. rattus, S. sobrinus, S. sanguis* and *S. oralis*, respectively. Whether the hemin binding protein of streptococci evolved from Dpr and Dps is not clear, but there is a possibility. In order to accurately identify the relationship between these proteins, further studies such as biochemical characterization of the Dpr protein and the hemin binding protein, protein structure confirmation, and cloning of hemin binding proteins genes are required.

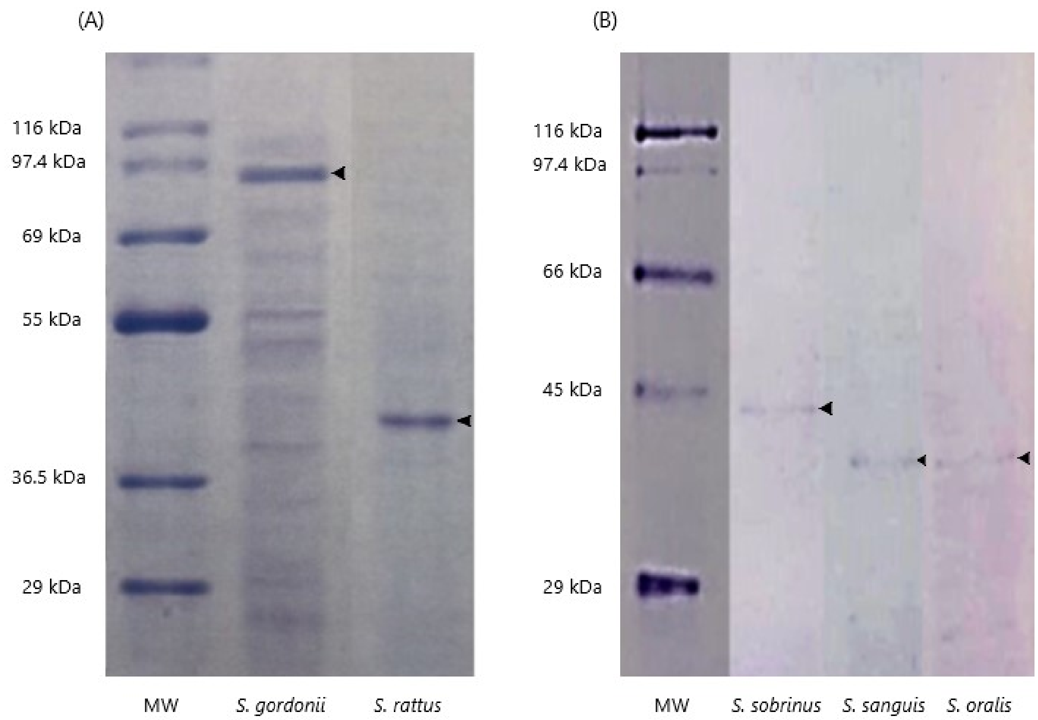

Fig. 1. SDS-PAGE analysis of hemin binding proteins of streptococci

Bacterial components which bound to hemin-agarose beads were prepared from streptococci. The samples were analyzed by SDS-PAGE with Coomassie Brilliant blue staining. (A) Lane 1 (molecular weight markers) represents the mass (kDa) of a protein molecule. Hemin binding protein was prepared from the S. gordonii DL1 (lane 2) and S. rattus Fa-1f (lane 3). (B) Lane 1 (molecular weight markers) represents the mass (kDa) of a protein molecule. Hemin binding protein was prepared from the S. sobrinus KN100 (lane 2) and S. sanguis KN107 (lane 3), S. oralis KN116 (lane 4).

4. CONCLUSION

In this paper, we confirmed the presence of hemin binding protein in streptococci by SDS-PAGE analysis using hemin agarose and showed that the proteins involved in hemin binding may be different if the bacterial species is different.

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