Induction of Hemagglutination by Recombinant Porphyromonas Gingivalis 40-kDa Outer Membrane Protein

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Abstract

Porphyromonas gingivalis, an important pathogen in periodontitis, possesses strong hemagglutinating activity. This is mediated by a variety of hemagglutinating molecules on its cell surface. We previously succeeded in cloning the gene encoding 40-kDa outer membrane protein (40-kDa OMP) from P. gingivalis 381. To clarify the pathological role of 40-kDa OMP, we examined its hemagglutinating activity. The recombinant 40-kDa OMP (r40-kDa OMP) was highly purified and used to raise a rabbit antiserum, which was then purified by r40-kDa OMP affinity chromatography. Although the r40-kDa OMP itself did not show hemagglutinating activity, a cross-linked polymeric form had strong activity. Finally, the affinity-purified anti-r40-kDa OMP antibody inhibited hemagglutination caused by P. gingivalis vesicles. These findings suggest that 40-kDa OMP is a novel hemagglutinin that plays an important role in the pathogenicity of P. gingivalis.

Introduction

Porphyromonas gingivalis has been implicated as an important pathogen in the development of periodontitis, a chronic inflammatory disease of periodontal tissues (1, 2). Adherence of bacteria to host tissue cells is a prerequisite for colonization and a causative factor in bacterial pathogenesis. In periodontal diseases, the colonization of periodontal pathogens on gingival tissues and the agglutination of erythrocytes (hemagglutination) are critical to the pathogenic process. P. gingivalis adheres strongly to both erythrocytes and epithelial cells (3), and protoheme, a product naturally released by erythrocytes, is essential for the growth of P. gingivalis (4). Therefore, hemagglutinating activity of P. gingivalis is necessary for colonization of gingival tissues and may also serve a nutritional function for the bacteria (5).

Specific bacterial surface hemagglutinin proteins act as virulence factors for a number of bacterial species, including P. gingivalis (3). We previously succeeded in cloning a 40-kDa outer membrane protein (40-kDa OMP) from P. gingivalis 381 by immunological screening with an antibody against an outer membrane preparation (6). We found that 40-kDa OMP is a co-aggregation factor for oral bacteria (7). In this study, we examined the pathogenic role of 40-kDa OMP in greater detail. Because polymeric forms of aggregating factors are usually required to bridge adjoining cells during hemagglutination, we examined the effect of chemically cross-linked recombinant 40-kDa OMP (r40-kDa OMP). We further characterized the effect of an antibody against r40-kDa OMP on hemagglutination caused by P. gingivalis vesicles.

Materials and Methods

Preparation of polymeric r40-kDa OMP

Using the method of Kawamoto et al. (12), r40-kDa OMP was purified to homogeneity from the recombinant clone. Chemical cross-linking was accomplished by incubating 13 mg of r40-kDa OMP in 2 ml of 0.1 M sodium hydrogen carbonate and 62.3 mg of carbodiimide hydrochloride (Peptide Institute Inc, Osaka, Japan). The mixture was stirred for 30 min at room temperature, after which it was dialyzed against PBS at 4 °C overnight. The dialyzed reaction mixture was centrifuged at 10,000 g for 10 min at 4 °C, and the supernatant fraction was used for hemagglutination experiments. Finally, to examine the cross-linking of r40-kDa OMP, a sample of the reaction was separated by 7% SDS-PAGE, after which proteins were stained with Coomassie Brilliant Blue R-250.
Preparation of the affinity purified mono-specific antibody

Rabbit antiserum against the purified r40-kDa OMP was obtained (7), and serum was passed through a diethylaminoethyl cellulose column (DE52, Whatman Ltd, Maidstone, England), and the eluant was applied to an immuno-affinity column (Glycosyl-hardgel; ICN ImmunoBiologicals Tokyo, Japan) containing purified r40-kDa OMP according to manufacture protocol. After extensive washing with PBS, the affinity purified mono-specific antibody against r40-kDa OMP (Af-Ab-OMP) was eluted with 0.2 M glycine-HCl buffer (pH 2.3) and dialyzed against PBS at 4 °C for overnight.

Hemagglutinating activity assay

P. gingivalis 381 vesicles were isolated as described by Grenier and Mayrand (13) with some modification as described by Hiratsuka et al. (7). Briefly, 50 μl of P. gingivalis vesicles or purified r40-kDa OMP was transferred into the wells of round-bottom 96-well microtiter plate after which 50 μl of 2% (v/v) rabbit erythrocytes were added. Following incubation for 15 min at 37 °C, hemagglutination was assessed by photographic observation. For inhibition experiments, the P. gingivalis vesicles were pre-incubated with Af-Ab-OMP for 1 h at 37 °C prior to the hemagglutinating assay.

Results and Discussion

In the current studies, we examined the pathological role of 40-kDa OMP by examining its effect on hemagglutination. Because hemagglutination typically requires polymeric forms of proteins to bridge two adjoining cells, we prepared chemically cross-linked r40-kDa OMP. The r40-kDa OMP was cross-linked using carbodiimide hydrochloride, and following the cross-linking reaction, a variety of protein bands larger than monomeric r40-kDa OMP were observed on SDS-PAGE gels (Fig. 1). In contrast, the monomeric, untreated r40-kDa OMP appeared as a single 40 kDa band. These results indicate that this procedure successfully generated polymeric forms of r40-kDa OMP. Ovalbumin conjugates were prepared as control for hemagglutinating activity caused by the cross-linking agents and process.

The hemagglutinating activity of r40-kDa OMP is shown in Fig. 2. Neither the monomeric r40-kDa OMP alone nor the ovalbumin conjugate induced hemagglutination. In contrast, the polymeric r40-kDa OMP strongly and dose-dependently induced hemagglutination.

We further examined the effect of a rabbit anti-40-kDa OMP antibody on the hemagglutinating activity of P. gingivalis vesicles. Because rabbit antisera contain non-specific antibodies, we prepared an affinity-purified monospecific antibody by affinity chromatography using immobilized r40-kDa OMP. As shown in Fig. 3, Af-Ab-OMP dose-dependently inhibited the hemagglutinating activity of P. gingivalis vesicles.

Collectively, these results indicate that polymers of 40-kDa OMP can mediate hemagglutination. Therefore, our results strongly suggest that 40-kDa OMP is a hemagglutinin.
on \textit{P. gingivalis} surfaces and that it is a virulence factor for \textit{P. gingivalis} infection of gingival tissues.

Several hemagglutinins have been cloned and sequenced from \textit{P. gingivalis} cells, including fimbrins, Hag family proteins, and the hemagglutinin-inducing proteases ptp, prtT, and Arg- and Lys- gingipains (9). Recently, we analyzed the nucleotide sequence of a gene encoding 40-kDa OMP (10). We found that the 40-kDa OMP gene (AB059658) is not related to fimbrins, Hag family proteins, and proteases. Thus, 40-kDa OMP appears to be a novel \textit{P. gingivalis} cell surface molecule with hemagglutinating activity.

It has recently been suggested that periodontal diseases may be related to susceptibility to certain systemic diseases including cardiovascular disease (11). Given that 40-kDa OMP appears to be a hemagglutinin, passive immunotherapy using the anti-40-kDa OMP may provide an approach for preventing periodontal and systemic diseases caused by \textit{P. gingivalis} through inhibitory effects on the colonization to gingival tissues and the binding to erythrocyte.

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