

## Characterization of Binding of *Streptococcus oralis* Glyceraldehyde-3-Phosphate Dehydrogenase to *Porphyromonas gingivalis* Major Fimbriae

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**Binding of *Streptococcus oralis* glyceraldehyde-3-phosphate dehydrogenase (GAPDH) to *Porphyromonas gingivalis* fimbriae was characterized via a biomolecular interaction analysis system. The interaction was specific, and the association constant value was  $4.34 \times 10^7 \text{ M}^{-1}$ , suggesting that *S. oralis* GAPDH functions as a dominant receptor for *P. gingivalis* and contributes to *P. gingivalis* colonization.**

Interaction of *Porphyromonas gingivalis*, which is a predominant periodontal pathogen, with early plaque-forming bacteria plays an important role with respect to colonization in periodontal pockets (4, 12, 13). *P. gingivalis* interacts with a variety of other oral gram-positive bacteria, including *Actinomyces naeslundii* (16), *Streptococcus gordonii* (5), *Streptococcus oralis* (7), and *Streptococcus sanguinis* (14); these intergeneric coaggregations may lead to the initial colonization of *P. gingivalis* in the oral cavity.

A series of studies demonstrated that *P. gingivalis* 381 strongly coaggregated with *S. oralis* ATCC 9811; moreover, its fimbriae were primarily responsible for the interaction, in which several domains of the carboxy terminus of fimbrillin participated (1). A recent paper regarding coadhesin of *S. oralis* by Maeda et al. (6) revealed that glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of *S. oralis* ATCC 9811 mediated binding to *P. gingivalis* 381 major fimbriae. GAPDH, which is a tetrameric enzyme of the glycolytic pathway, is responsible for the phosphorylation of glyceraldehyde-3-phosphate, which leads to generation of 1,3-bisphosphoglycerate (15). In addition to its enzymatic activity, GAPDH reportedly possesses multiple binding activities. Pancholi and Fischetti (11) found that the GAPDH of group A streptococci binds to fibronectin, lysozyme, and the cytoskeletal proteins myosin and actin; they noted that it may function in the colonization of those bacteria. In the present study, kinetic interaction of GAPDH of *S. oralis* ATCC 9811 with *P. gingivalis* 381 fimbriae was analyzed based on surface plasmon resonance spectroscopy with a biomolecular interaction analysis system (BIAcore).

*S. oralis* ATCC 9811 was maintained as frozen stocks and was cultured in brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) for 15 h at 35°C in air. Bacterial cells were harvested by centrifugation (High Speed Refrigerated Centrifuge SRX-201; Tomy Seiko Co. Ltd., Tokyo, Japan)

at  $5,000 \times g$  for 30 min at 4°C; they were subsequently washed three times with 20 mM phosphate buffer supplemented with 0.15 M NaCl (phosphate-buffered saline [PBS] [pH 6.0]) and were suspended in the same buffer.

*S. oralis* ATCC 9811 GAPDH was purified by mild ultrasonication, 3-[(3-cholamidopropyl)-dimethylammonio]-1-propanesulfonate treatment, ammonium sulfate precipitation, and chromatography with an affinity column coupled with *P. gingivalis* recombinant fimbrillin (rFimA) as reported previously (6). *P. gingivalis* 381 rFimA and *S. oralis* ATCC 9811 recombinant GAPDH (rGAPDH) were prepared in accordance with the methods previously reported (6, 8). Interactions between *P. gingivalis* rFimA and native and recombinant *S. oralis* GAPDH were analyzed with a BIAcore 2000 apparatus (Uppsala, Sweden). The carboxymethylated dextran matrix on the CM5 sensorchip (BIAcore) was activated with *N*-hydroxysuccinimide and *N*-ethyl-*N'*-[(3-dimethylamino)-propyl]-carbodiimide hydrochloride (1:1) at a flow rate of 5  $\mu\text{l}/\text{min}$  at 37°C. Native GAPDH or rGAPDH (20  $\mu\text{g}/\text{ml}$ ) in 10 mM sodium acetate buffer (pH 4.8) was immobilized on the matrix, because they were immobilized most on the matrix at pH 4.8 among tested pHs. Although the effect of the pH on the protein conformation was not determined in this study, we confirmed that the coaggregation activity between *P. gingivalis* and *S. oralis* was almost the same at pH 4.8 as it was at pH 6.0. To equalize the amount (in moles) of the immobilized proteins, the increase in resonance units (RU) produced by immobilization was manually set at  $650 \times$  (molecular mass of immobilized protein [40 kDa]/molecular mass of fimbrillin [41 kDa]) RU according to the manufacturer's manual. Excess active sites of the matrix were blocked with 1 M ethanolamine-HCl and washed with 10 mM NaOH. All materials were dissolved in 10 mM PBS (pH 6.0), which also served as a running buffer in the experiments. *P. gingivalis* rFimA was injected across the active CM5 (GAPDH) and an empty control CM5 surface at a flow rate of 20  $\mu\text{l}/\text{min}$  at 37°C. Binding of rFimA was monitored and presented as RU in a sensorgram. One-thousand RU corresponded to a change in the surface concentration of 1  $\text{ng}/\text{mm}^2$  on the sensory chip. At the end of each run the surface was regenerated by successive injections of 10 mM NaOH. Specific

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TABLE 1. Kinetic parameters for *P. gingivalis* rFimA binding to immobilized *S. oralis* native GAPDH and rGAPDH<sup>a</sup>

Ligand	$k_{\text{ass}}$ ( $\text{M}^{-1}\text{s}^{-1}$ )	$k_{\text{diss}}$ ( $\text{s}^{-1}$ )	$K_a$ ( $\text{M}^{-1}$ )
<i>S. oralis</i> native GAPDH	$2.65 \times 10^4$	$6.11 \times 10^{-4}$	$4.34 \times 10^7$
<i>S. oralis</i> rGAPDH	$7.41 \times 10^4$	$1.10 \times 10^{-3}$	$6.75 \times 10^7$

<sup>a</sup> *P. gingivalis* rFimA was the analyte in each case.

binding profiles of rFimA to the immobilized GAPDH were obtained following subtraction of the response signal from the control surface. Analysis of these kinetic parameters was conducted with BIAevaluation 3.1, a software package (BIAcore), according to the operator's manual. Protein concentration of the samples was determined with a bicinchoninic acid protein assay kit (Pierce, Rockford, Ill.) utilizing bovine serum albumin as a standard.

The association rate constants ( $k_{\text{ass}}$ ), dissociation rate constants ( $k_{\text{diss}}$ ), and equilibrium association constants ( $K_a = k_{\text{ass}}/k_{\text{diss}}$ ) of native GAPDH and rGAPDH of *S. oralis* ATCC 9811 for binding of *P. gingivalis* rFimA are summarized in Table 1. The representative sensorgrams exhibited in Fig. 1 revealed that the resonance response reflecting *P. gingivalis* rFimA-*S. oralis* native GAPDH or rGAPDH interaction occurred in an analyte concentration-dependent manner. *S. oralis* native GAPDH and rGAPDH were characterized by significantly high  $k_{\text{ass}}$  values ( $2.65 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$  and  $7.41 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$ , respectively), which indicated rapid association with rFimA. At the dissociation phase, *S. oralis* native GAPDH and rGAPDH  $k_{\text{diss}}$  values were  $6.11 \times 10^{-4} \text{ M s}^{-1}$  and  $1.1 \times 10^{-3} \text{ M s}^{-1}$ , respectively, which were representative of high stabilities. Total affinities were presented as  $K_a$ .  $K_a$  values of native GAPDH and rGAPDH with rFimA ( $4.34 \times 10^7 \text{ M}^{-1}$  and  $6.75 \times 10^7 \text{ M}^{-1}$ , respectively) demonstrated high affinity.

$K_a$  values of many antibody-protein antigen interactions occur within the range  $10^6$  to  $10^{10} \text{ M}^{-1}$  (3). In this study,  $K_a$  values for *P. gingivalis* rFimA and *S. oralis* native GAPDH and rGAPDH were  $4.34 \times 10^7 \text{ M}^{-1}$  and  $6.75 \times 10^7 \text{ M}^{-1}$ , respectively, which indicated that these interactions were specific. *P. gingivalis* fimbriae reportedly bind to a variety of components, such as epithelial cells, fibroblasts, components of saliva, and several extracellular matrix proteins (2). Nakamura et al. (9) documented the following  $K_a$  values between *P. gingivalis* fimbriae and extracellular matrix proteins:  $2.15 \times 10^6 \text{ M}^{-1}$  (laminin),  $2.16 \times 10^6 \text{ M}^{-1}$  (fibronectin),  $2.26 \times 10^6 \text{ M}^{-1}$  (thrombospondin),  $2.76 \times 10^6 \text{ M}^{-1}$  (type I collagen),  $3.08 \times 10^6 \text{ M}^{-1}$  (elastin), and  $3.79 \times 10^6 \text{ M}^{-1}$  (vitronectin). In addition, Amano (2) reported  $K_a$  values between *P. gingivalis* fimbriae and acidic proline-rich protein, basic proline-rich glycoprotein, statherin, fibrinogen, and hemoglobin of  $1.63 \times 10^6 \text{ M}^{-1}$ ,  $1.62 \times 10^6 \text{ M}^{-1}$ ,  $1.48 \times 10^6 \text{ M}^{-1}$ ,  $2.16 \times 10^6 \text{ M}^{-1}$ , and  $2.43 \times 10^6 \text{ M}^{-1}$ , respectively. The  $K_a$  value obtained in the present experiment was higher than those of the aforementioned interactions. Consequently, the interaction of *P. gingivalis* fimbriae with *S. oralis* GAPDH is considered fairly strong.

Streptococci and actinomycetes are believed to be the major initial colonizers of the pellicle on the tooth surface; furthermore, interactions between these bacteria and their substrata aid the establishment of the early biofilm community (4). Nyvad and Kilian (10) reported that *S. oralis*, *S. sanguinis*, and *S.*

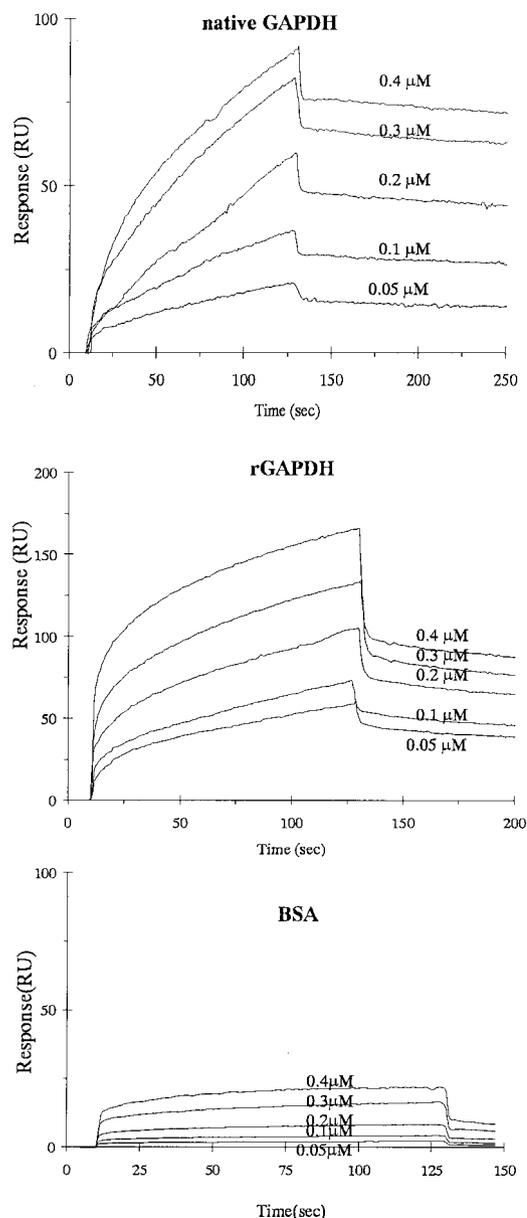


FIG. 1. Sensorgrams of *P. gingivalis* rFimA binding to immobilized *S. oralis* native GAPDH, rGAPDH, and bovine serum albumin in kinetic studies. *P. gingivalis* rFimA solution was injected over *S. oralis* native GAPDH, rGAPDH, or bovine serum albumin (BSA) on the sensor chip at various concentrations (0.05 to 0.4  $\mu\text{M}$ ).

*mitis* represent 60 to 90% of cultivable streptococci within the first 4 h of plaque formation. In the present study, *S. oralis* ATCC 9811 GAPDH demonstrated high affinity with *P. gingivalis* fimbriae. Extensive reports have shown that the binding interaction of *P. gingivalis* and streptococci is multimodal; however, considering the conservation of streptococcal GAPDHs (6) and the high affinity of *S. oralis* GAPDH binding to *P. gingivalis* fimbriae, early plaque-forming streptococcal GAPDHs may contribute to *P. gingivalis* colonization in periodontal pockets.

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