Binding of catalase by *Gardnerella vaginalis*

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Abstract

Previous work has demonstrated that *Gardnerella vaginalis* can utilize catalase as a sole source of iron. In this study, the interaction between *G. vaginalis* cells and catalase was investigated. *G. vaginalis* cells were shown to bind digoxigenin (DIG)-labeled catalase using a solid phase dot blot assay. An increase in catalase binding was observed from cells grown under iron-restrictive conditions. Western blot analysis of *G. vaginalis* proteins resulted in the detection of a putative catalase-binding protein with an estimated molecular mass of 128 kDa. The 128-kDa catalase-binding protein was not detected from intact *G. vaginalis* cells treated with trypsin prior to Western blot analysis suggesting this protein may be surface-exposed. © 2000 Federation of European Microbiological Societies. Published by Elsevier Science B.V. All rights reserved.

Keywords: Catalase-binding protein; Surface-exposed protein; Iron; Gram-positive

1. Introduction

Iron is an essential growth factor required by virtually all bacteria. The acquisition of iron can play an important role in the virulence potential for many bacterial pathogens [1,2]. However, free iron is found in limited amounts in the human host. The majority of iron is bound by high-affinity iron-binding proteins such as transferrin or lactoferrin or sequestered in compounds such as heme, ferritin, and hemoglobin. As a result, pathogenic bacteria have adapted to this iron-limiting environment by developing high-affinity iron acquisition mechanisms to obtain iron from host sources. One mechanism is the utilization of low-molecular-mass, high-affinity iron chelators known as siderophores which remove iron from carrier molecules [3]. A second mechanism is the utilization of specific cell surface receptors to directly bind iron-containing compounds such as heme, hemoglobin, lactoferrin, and transferrin [1,2].

*Gardnerella vaginalis* is the predominant bacterium associated with bacterial vaginosis (BV), a common disorder of women of reproductive age [4]. BV has been shown to be a significant risk factor for upper genital tract infections, which can result in adverse outcomes of pregnancy, and also increases the risk of HIV infection [5–7]. In addition to its association with BV, the Gram-positive *G. vaginalis* has been detected in or isolated from other infections including intraamniotic, chorioamniotic, intrauterine, and urinary tract infections [4]. Furthermore, recent studies by Hashemi et al. demonstrated that *G. vaginalis* cell lysates could stimulate HIV-1 gene expression in human cell cultures suggesting *G. vaginalis* may play a role in the increased rate of HIV transmission in BV patients [8]. However, little is known about the pathogenic mechanisms of *G. vaginalis*.

There is little information about iron acquisition by *G. vaginalis* and its role in the virulence potential of this organism. Previous work from our laboratory demonstrated that *G. vaginalis* could utilize several iron-containing compounds as a sole source of iron, including catalase [9]. However, it is not known how *G. vaginalis* acquires iron from catalase. In this study, the interaction between *G. vaginalis* cells and catalase was examined. *G. vaginalis* cells were shown to bind catalase and a 128-kDa *G. vaginalis* catalase-binding protein (Cbp) was detected.

2. Materials and methods

2.1. Bacterial strains, media, growth conditions, and chemicals

*G. vaginalis* strain 594 (ATCC 14018) and *G. vaginalis*
DIG (DIG-3-0-succinyl-
-bu¡ered saline (PBS), pH 7.4, was incubated with 100
tions. Brie£y, 2 mg of catalase dissolved in phosphate-
olis, IN, USA) according to the manufacturer’s instruc-
 DIG Protein Labeling kit (Roche Diagnostics, Indianap-
2.2. Labeling of catalase with digoxigenin (DIG)

Labeling of catalase with DIG was performed using the
DIG Protein Labeling kit (Roche Diagnostics, Indianapolis, IN, USA) according to the manufacturer’s instruc-
Briefly, 2 mg of catalase dissolved in phosphate-
trypsin, the protease inhibitor phenylmethylsulfonyl fluo-
chia coli
strain DH5
2.3. Solid-phase dot blot binding assay

Twenty-four-hour cultures of G. vaginalis strains grown
on iron-replete media (BMS) were resuspended in BMS
broth and cultures grown on iron-restrictive media (BMS
supplemented with 100 µM deferoxamine mesylate) were
resuspended in BMS broth with 100 µM deferoxamine
mesylate at a concentration of 10^9 cells ml^-1. 10 µl of
the cell suspensions was then vacuum-blotted onto Immo-
bilon-NC nitrocellulose filters (Millipore, Bedford, MA, USA). After drying for 2 h at 37°C, the filters were probed with DIG-labeled catalase (10 µg ml^-1 final concentration) for 1 h, washed with TBS, and then incubated with anti-DIG-POD antibody
(1:25000 final dilution) for 1 h at room temperature. After
the unbound antibody was removed by washing, the blots
were developed utilizing the chemiluminescence detection
kit as described above. To determine specificity of the
binding activity of the 128-kDa protein, Western blot
analysis of G. vaginalis proteins was performed as de-
scribed above with the exception that the nitrocellulose
filters were preincubated with unlabeled catalase (final
centration 100 µg ml^-1) or hemin (final concentration
10 µg ml^-1) for 1 h prior to the addition of DIG-labeled
catalase.

2.5. Proteolytic treatment of G. vaginalis cells

One milliliter of G. vaginalis cells (10^8 ml^-1) was incub-
bated with trypsin (50 µg ml^-1) for 15 min at 37°C. After
washing with BMS broth to remove the trypsin, the cells
were resuspended (10^9 ml^-1) in BMS broth containing
PMSF at a final concentration of 1 mM and lysed with
Laemmli SDS-PAGE sample buffer. The cell proteins were
then analyzed by SDS-PAGE and Western blot analysis as
described above.

3. Results and discussion

3.1. Binding of catalase by G. vaginalis cells

G. vaginalis inhabits an environment which contains sev-
eral host compounds, including catalase, that could serve
as potential iron sources. We have previously shown that
G. vaginalis could utilize catalase as a sole source of iron
[9]. G. vaginalis expresses a 60-kDa cytolysin which can
lyse erythrocytes, neutrophils, and endothelial cells [13].
Presumably, catalase is released upon lysis of host cells.
The utilization of catalase as an iron source has also been
demonstrated in other bacteria including Haemophilus
ducreyi [14] and Streptococcus pyogenes [15].
The mechanism by which G. vaginalis utilizes catalase is
3.2. Detection of a 128-kDa Cbp

Since *G. vaginalis* cells could utilize and directly bind catalase, Western blot analysis of *G. vaginalis* proteins was utilized to identify any potential *G. vaginalis* protein(s) involved in the binding of catalase. Proteins from lysates obtained from *G. vaginalis* cells grown under iron-restrictive conditions were separated via SDS-PAGE, electroblotted onto nitrocellulose filters, and probed with DIG-labeled catalase. Fig. 2 shows that a Cbp with an estimated molecular mass of 128 kDa was detected from *G. vaginalis* strains 594 and 317 (Fig. 2, lanes 2 and 4) suggesting this activity may be iron-regulated. As a negative control, no catalase binding was observed by *E. coli* DH5αMCR (Fig. 1, lane 5). In the Gram-positive bacterium *Corynebacterium diphtheriae*, the regulation of siderophore production and the diphtheria toxin is mediated by iron and the DtxR protein, an iron-dependent repressor of gene expression [16]. However, whether the catalase-binding activity by *G. vaginalis* is mediated by a DtxR homolog remains to be determined.

![Image 1](https://example.com/image1)

**Fig. 1.** Binding of DIG-labeled catalase by *G. vaginalis* cells. *G. vaginalis* cells were electroblotted onto nitrocellulose filters and probed with DIG-labeled catalase as described in Section 2. The figure shows representative results of the experiment done in triplicate. 1, *G. vaginalis* 594 grown under iron-replete conditions; 2, *G. vaginalis* 594 grown under iron-restrictive conditions; 3, *G. vaginalis* 317 grown under iron-replete conditions; 4, *G. vaginalis* 317 grown under iron-restrictive conditions; 5, *E. coli* DH5αMCR.

![Image 2](https://example.com/image2)

**Fig. 2.** Detection and characterization of a 128 kDa *G. vaginalis* Cbp via Western blot analysis. *G. vaginalis* proteins from whole cell lysates were electroblotted onto nitrocellulose and probed with DIG-labeled catalase. Equal amounts of protein (about 35 μg) were loaded into each lane for SDS-PAGE. Odd numbered lanes, *G. vaginalis* 594; even numbered lanes, *G. vaginalis* 317. Lanes 1 and 2, *G. vaginalis* grown under iron-restrictive conditions (BMS supplemented with 100 μM deferoxamine mesylate); lanes 3 and 4, Western blot filters precultivated with unlabeled catalase prior to exposure of DIG-labeled catalase; lanes 5 and 6, Western blot filters precultivated with hemin prior to exposure of DIG-labeled catalase; lanes 7 and 8, proteins from intact *G. vaginalis* cells treated with trypsin prior to lysis and SDS-PAGE. M, molecular mass standards in kDa. The figure shows representative results of the experiment done in triplicate.

In conclusion, *G. vaginalis* cells were shown to directly bind catalase. Additionally, a 128-kDa surface exposed Cbp was detected from *G. vaginalis* cells grown under iron-limiting conditions. Future work will focus on determining what role the *G. vaginalis* Cbp plays in the acquisition of iron from catalase, the regulation of the catalase-binding activity, and the identification and characterization of the gene encoding this Cbp.

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**References**
