

Achromopeptidase for Lysis of Anaerobic Gram-Positive Cocci

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Achromopeptidase, which has potent bacteriolytic activity for most of the gram-positive aerobic bacteria, was for the first time used for the lysis of anaerobic cocci. Most of the lysozyme-resistant gram-positive anaerobic cocci were lysed with this new enzyme. *Peptococcus magnus* was the only organism tested resistant to achromopeptidase. *P. saccharolyticus* was quite unusual because it was very sensitive to both achromopeptidase and lysozyme.

There are many gram-positive bacteria that are resistant to lysozyme or other existing bacteriolytic enzymes. Achromopeptidase (recently supplied by Wako Pure Chemical Industries, Ltd., Japan) has potent bacteriolytic activity for many gram-positive organisms (3, 6, 7). This enzyme can lyse *Staphylococcus aureus* and *Streptococcus faecalis*, which are resistant to lysozyme (3, 6). However, as it has not yet been applied for the lysis of anaerobic cocci, which are mostly resistant to lysozyme, the sensitivity of anaerobic cocci to achromopeptidase is not known.

Enzymatic or chemical disruption effective for most of the species of anaerobic cocci has not yet been described. We used this enzyme for the lysis of anaerobic cocci and noticed that almost all of the gram-positive anaerobic cocci were effectively lysed with this enzyme. In this paper, we also compare the lytic activity between achromopeptidase and lysozyme.

MATERIALS AND METHODS

Reference strains and 157 strains of human clinical isolates were used in this study. Strains frozen at -90°C in 20% milk were thawed and inoculated into a Gifu anaerobic medium (GAM) (Nissui Co., Ltd., Japan). After being checked for purity, the organisms were inoculated into 20 ml of GAM broth and incubated at 37°C for 18 to 24 h in an anaerobic chamber (85% nitrogen; 10% carbon dioxide; 5% hydrogen). Cells were harvested by centrifugation and washed twice with saline. After washing, cells were suspended in 0.01 M Tris buffer (pH 8.0) or 0.1 M phosphate buffer (pH 6.0). Absorbance of each suspension was adjusted to approximately 0.6 at 600 nm. Achromopeptidase (TBL-1, purified) was added to the cells suspended in the Tris buffer, and lysozyme (grade 1, Sigma Chemical Co., St. Louis, Mo.) was added to the cells in the phosphate buffer to the required concentrations. The mixtures were kept at 37°C , and the changes in absorbance at 600 nm were recorded.

RESULTS

The bacteriolytic activities of achromopeptidase and lysozyme are shown in Fig. 1. Reference strains of anaerobic cocci, except for *Peptococcus saccharolyticus*, were resistant to both achromopeptidase and lysozyme at a concentration of 100 U/ml. At 500 U of achromopeptidase per ml, *P. asaccharolyticus*, *P. indolicus*, and *Peptostreptococcus micros* were lysed, but it took about 2 h to decrease the turbidity to half the original density. These bacteria were not lysed with lysozyme of the same concentration. At 1,000 U of achromopeptidase per ml, *P. asaccharolyticus*, *P. indolicus*, and *Peptostreptococcus micros* were rapidly lysed. Within 15 min, the turbidity of the bacterial suspension decreased to half the original concentration. At the same concentration, lysozyme could not lyse reference anaerobic cocci, except for *P. saccharolyticus*. *P. saccharolyticus* was as sensitive to achromopeptidase as *S. aureus* and *S. faecalis* were.

The results of lysis of our stock strains with achromopeptidase at a concentration of 1,500 U/ml are shown in Table 1. Although most strains of anaerobic cocci, except for *P. magnus* and some strains of *P. prevotii*, were sensitive to this enzyme, it took 2 h to decrease the turbidity to half the original concentration in many cases.

DISCUSSION

Achromopeptidase has potent bacteriolytic activity for most gram-positive bacteria that have been examined (6). *S. aureus* and *S. faecalis*, which are lysozyme resistant, are rapidly lysed with achromopeptidase at a low concentration. Turbidity of bacterial suspensions decreases markedly within 15 min when they are incubated with 500 U of achromopeptidase per ml. However, at the same concentration, lysis of

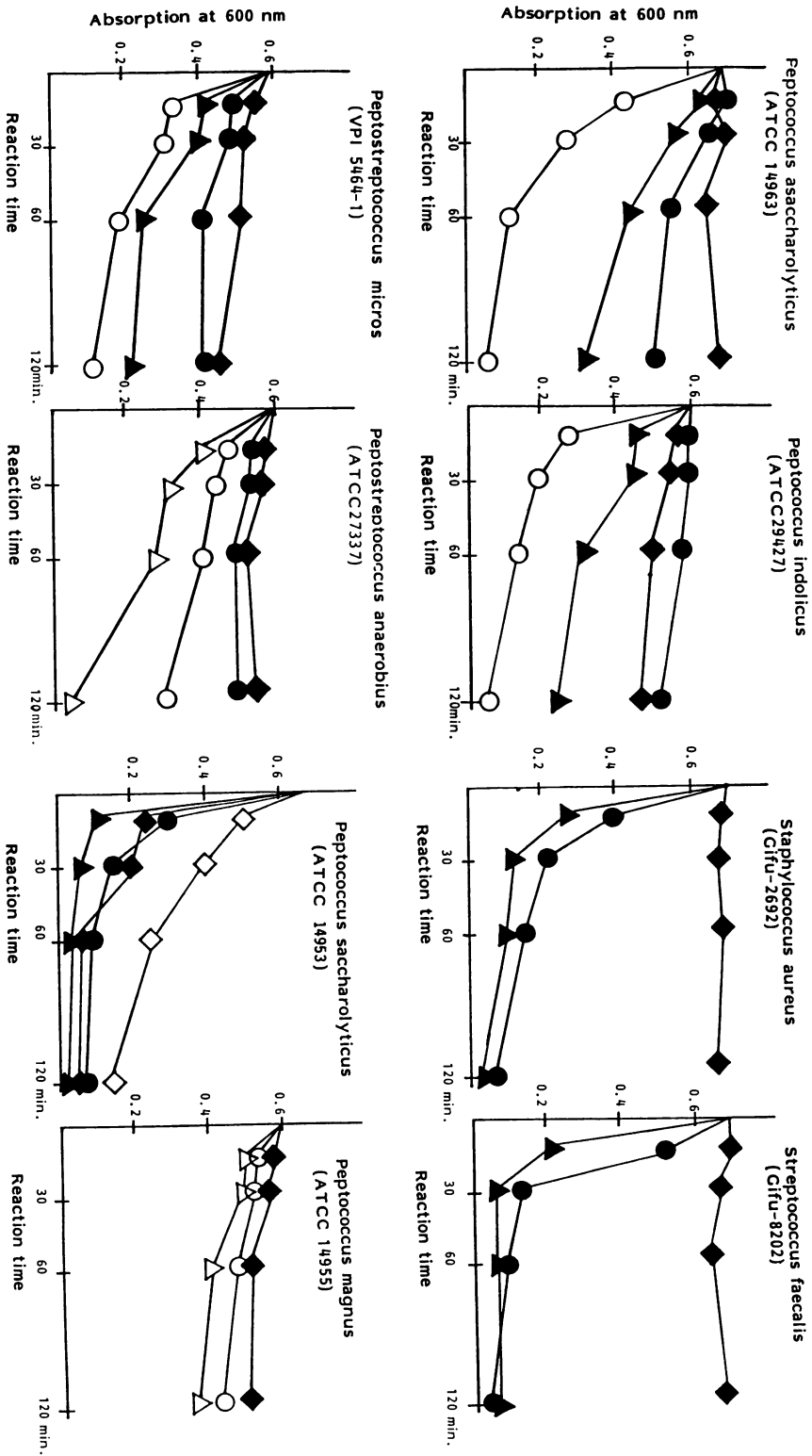


FIG. 1. Lysis of reference strains with achromopeptidase. Symbols (concentrations in U/ml): \diamond , Lysozyme, 500; \blacklozenge , lysozyme, 1,500; \bullet , achromopeptidase, 100; \blacktriangle , achromopeptidase, 500; \circ , achromopeptidase, 1,000; \triangle , achromopeptidase, 1,500.

TABLE 1. Cell lysis of anaerobic cocci with achromopeptidase

Organism	No. of strains tested	No. of strains with the following sensitivity to achromopeptidase: ^a				
		+++	++	+	±	-
<i>Peptococcus asaccharolyticus</i>	34	18	12	4	0	0
<i>Peptococcus indolicus</i>	4	4	0	0	0	0
<i>Peptococcus prevotii</i>	28	11	7	7	3	1
<i>Peptococcus magnus</i>	47	7	3	14	13	10
<i>Peptococcus saccharolyticus</i>	5	5	0	0	0	0
<i>Peptostreptococcus micros</i>	6	4	2	0	0	0
<i>Peptostreptococcus anaerobius</i>	17	9	4	4	0	0
<i>Peptostreptococcus productus</i>	2	2	0	0	0	0
<i>Peptostreptococcus parvulus</i>	2	2	0	0	0	0
<i>Coprococcus catus</i>	2	2	0	0	0	0
<i>Ruminococcus albus</i>	3	3	0	0	0	0
<i>Gaffkya anaerobia</i>	8	4	3	1	0	0
<i>Sarcina ventriculi</i>	1	1	0	0	0	0

^a Symbols: +++, decrease of absorption at 600 nm is more than 80% in 1 h; ++, decrease from 40 to 80% in 1 h; +, decrease from 40 to 80% in 2 h; ±, decrease from 10 to 40% in 2 h; -, decrease less than 10% in 2 h.

anaerobic cocci, except for *P. saccharolyticus*, was either not achieved or delayed. Many anaerobic cocci were slowly lysed, even at concentrations of 1,000 or 1,500 U/ml. These results suggest greater resistance of anaerobic cocci than of *S. aureus* and *S. faecalis* to lysis with achromopeptidase. However, the fact that reference strains (except for *P. saccharolyticus*) were not lysed with lysozyme even at a concentration of 1,500 U/ml indicates that anaerobic cocci are more sensitive to achromopeptidase than to lysozyme.

P. saccharolyticus was unusually sensitive to achromopeptidase among the reference strains used in this study. This organism is also characterized by high sensitivity to lysozyme, unlike other reference strains of anaerobic cocci. Some strains of *P. saccharolyticus* develop an aerobic nature (1). Schleifer and Nimmerman reported

that the cell wall peptidoglycan constitution of this species differed from that of other species of *Peptococcus* (8). Furthermore, Kilpper and co-workers reported that *P. saccharolyticus* was more closely related to *Staphylococcus* species than to *Peptococcus* species from the results of DNA-DNA homology experiments (4). The same workers recently proposed the transfer of *P. saccharolyticus* to *Staphylococcus saccharolyticus* (Zentralbl. Bakteriologie, Parasitenkunde, Infektionskrankheiten, Hygiene, Abteilung 1, Originalreihe C 2:324-331). Our laboratory analysis also confirmed these results (unpublished data).

Most anaerobic cocci are resistant to existing bacteriolytic enzymes and chemical analysis. This posed one of the obstacles for studies on the intracellular enzymes, metabolism, and nucleic acids of these organisms. The only method commonly used for effective lysis of anaerobic cocci in our laboratory had been a physical disruption method with a CO₂ bead shaker (B. Braun, West Germany). By this method, however, nucleic acid is fragmented into uneven small pieces with inefficient recovery of nucleic acid. This new enzyme permits effective lysis of anaerobic cocci and extraction of nucleic acid, and it contributes to genetic studies (2).

Detailed studies of this enzyme have been recently published (5).

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