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Additional Properties of a Bacteriocin-like Agent (Acnecin) of *Propionibacterium acnes*

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Summary

Purified acnecin showed that it exerted bacteriostatic activity against susceptible indicator cells without adsorption on the cells, and its activity lost by treatment with Na-periodate or lysozyme. Acnecin inhibited also production of an extracellular enzyme (Ribonuclease) of acnecin-susceptible strain.

INTRODUCTION

Few reports have been published on the antagonistic substances produced by the genus *Propionibacterium*. Ko et al.[7] demonstrated the presence of an extracellular bacteriocin (propionicin) from *P. avidum* and showed that propionicin inhibited growth of many strains of *P. acnes, P. granulosum,* and *P. avidum.* However, the properties of propionicin was not discussed precisely. Holland et al.[6] reported intergeneric and intrageneric inhibition between strains of *P. acnes* and Micrococcaceae. They found a low frequency of inhibition of *Staphylococcus epidermidis* by *P. acnes* (1.1%) and a higher frequency of inhibition of *P. acnes* by Micrococcaceae (9.5%) and *P. acnes* (40.8%).Purification and characterization of a bacteriocin-like agent from *P. acnes,* desiganated "acnecin", has been described in a previous paper[3] from our laboratory. Acnecin exerted a bacteriostatic effect on susceptible microorganisms and was heat labile, destroyed by proteolytic enzymes, and contained carbohydrates as component. It exhibited extremly narrow range of inhibitory spectrum. In the present paper some additional properties of acnecin concerning to its interaction with the target cells and its biochemical nature are reported.

MATERIALS AND METHODS

Conditions for cultivation of microorganisms, purification of acnecin, and determination of acnecin activity by bioassay were described earlier.[3] Indicator strain used for the bioassay was *P. acnes* strain EXC-1 throughout the experiments. Ribonuclease (RNase) activity in the culture supernatant of EXC-1 was estimated by measurement of increase of acid-soluble products from yeast RNA (Sigma Chem. Co.) by the enzyme photometrically at 260 nm. One unit of RNase activity per milliliter was defined as the amount of which increased 0.1 A_{260} unit per 60 min.

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RESULTS AND DISCUSSION

Interaction of acnecin with the indicator cells

Adsorption tests of acnecin to the indicator cells was carried out essentially according to the method described by Dajani and Wannamaker.[1] To 1.0 g (wet weight) of the indicator cells, 5 ml of a dilute solution of acnecin (7 U/ml) in 0.05 M phosphate buffer (pH 7.0) was added and incubated at 37°C for 2 hr. After the removal of cells by centrifugation, no lowering of acnecin activity in the supernatant solution was observed. These findings indicate that acnecin inhibits growth of the target cells without adsorbing to their cell surfaces as has been demonstrated in staphylococcal staphylococcin,[5] *Clostridium perfringens* bacteriocin,[8] streptococcal viridin,[2] and streptococcal sanguicin.[4] It is noteworthy that these four bacteriocins and acnecin are bacteriostatic to the susceptible cells. This may suggest also that a class of a bacteriostatic bacteriocin lacks an ability to adsorb to the target cells.

No morphological alterations in the acnecin-treated indicator cells were detected, as far as observed with a light microscope. The indicator cells were treated with 640 U/ml acnecin for 5 hr at 37°C, and the cells were washed thoroughly with 0.05 M phosphate buffer (pH 7.0) containing 0.15 M saline. Then the cells were inoculated into the broth medium consisting of 3.7% brain heart infusion supplemented with 0.3% yeast extract and incubated anaerobically. When the growth was monitored turbidimetrically, there was no difference in the growth rate between acnecin-treated cells and control cells without treatment. Whereas in the presence of acnecin (640 U/ml), the indicator strain never grew. Therefore, acnecin molecules must coexist with the target cells to exhibit its inhibitory activity.

Necessity of carbohydrate moiety for the activity

Acnecin contains 3.3% (w/w) carbohydrates.[3] Whether the carbohydrate moiety is necessary for expression of the activity was investigated using carbohydrate-oxidizing reagent, Na-periodate or mucolytic enzyme, lysozyme. Acnecin (640 U/ml) was incubated with Na-periodate (Nakarai Chem. LTD) at a concentration of 10 mM in 0.2 M acetate buffer (pH 4.8) at 4°C for 5 hr in the dark. The reaction mixture was shaken at intervals of 1 hr. The residual activity in the mixture solution was assayed directly without removal of the reagent. Similarly, the effect of lysozyme (Sigma Chem. Co.) was tested with incubation at a concentration of 0.8 mg/ml for 30 min in 0.05 M phosphate buffer (pH 7.0). Treatment with the both reagents caused complete loss of the activity (Table 1). In these

Table 1 : Effect of Na-periodate or lysozyme on acnecin

Treatments with	Activity (U/ml) Initial	Residual
Na-periodate (10 mM) for 5 hr at 4°C	640	0
0.2 M acetate buffer (pH 4.8) for 5hr at 4°C	640	640
Lysozyme (0.8 mg/ml) for 30 min at 37°C	640	0
0.05 M phosphate buffer (pH 7.0) for 30 min at 37°C	640	640

experiments, buffer control showed no effect on the activity. Since another control experiments with the indicator cells treated with 10 mM Na-periodate for 5 hr at 4°C or with 0.8 mg/ml lysozyme for 30 min at 37°C showed also no alteration in the sensitivity to acnecin, the following possibility was ruled out ; the indicator cells which had contact with Na-periodate or lysozyme in the course of assay of the activity on agar plates resulted in inability to accept acnecin molecules, even if acnecin was intact. Therefore, acnecin is truely sensitive to the both reagents and thus the carbohydrate moiety as the protein one, is essential for the activity.

Effect of acnecin on RNase production

P. acnes is known to prodrce extracellular RNase.[9] To study the effect of acnecin on macromolecular metabolism, effect on RNase production, for insctance, was examined, using strain EXC-1. Acnecin was added to the culture at 3 days and 5 days after inoculation at a concentration of 320 U/ml. The cultivation was continued for a further 4 days and 2 days, respectively, and RNase activity in the culture supernatant was assayed at intervals. As illustrated in Fig. 1, RNase detected at 2 days and the production continued linearly for 6 days in the control culture. However, marked inhibition of RNase production occurred after addition of acnecin. A slight increase of RNase activity in the acnecin-treated culture may reflect partial inactivation of acnecin during cultivation. These results also suggest that *P. acnes* EXC-1 could not produce RNase in a resting state, since acnecin does not permit multiplication of susceptible cells. However, it is still obscure that acnecin inhibited the synthesis of the RNase protein or the secretion of it into the sorrounding medium or the both.

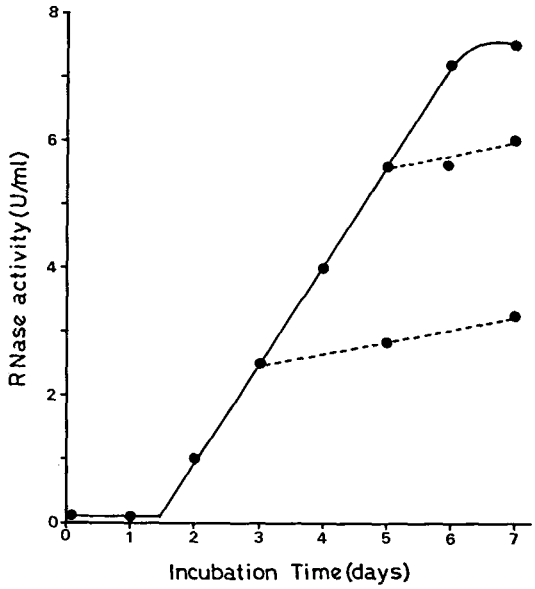

Fig. 1 : Effect of acnecin on RNase production of *P. acnes* strain EXC-1.
●——● control culture;
●- - -● acnecin-treated culture

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