PD-5

Effect of the ferric cation on thermostable direct hemolysin (TDH) produced by *Vibrio cholerae* non-O1 isolated from sea water

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Introduction

Vibrio cholerae non-O1 has been reported to cause food borne gastroenteritis. This strain produces thermostable direct hemolysin (NAG-rTDH) similar with that of *Vibrio parahaemolyticus*. As a result of rabbit ligated intestinal loop test, TDH caused fluid accumulation and is considered to be the major virulent factor. Hemolysis by TDH has been reported to cause colloid osmotic lysis of erythrocytes. In our laboratory, we tried to observe microscopical picture of sheep erythrocytes by TDH treatment in presence or absence of various divalent cations, and investigate the effects on hemolytic activity by exposing ferric cations to TDH changing concentration of TDH and ferric cation. Futhermore, we studied the effect on hemolytic activity by adding divalent cations with EGTA.

Materials and methods

TDH purification

TDH was purified by the modified method of Oh et al. (1993). Modified marine broth (MMB) was used for strain culture.

Hemolytic activity assay

Sheep erythrocytes were used for hemolytic activity assay, which was carried out by the method of Oh et al. (1993).

Results

We isolated *Vibrio cholerae* non O1 strains from sea water. Its hemolytic activity was identified on sheep blood plate and cultured by modifited marine broth medium adding 3% glycerol. We obtained partially purified hemolysin by using phenyl sepharose high performance column 16/10 (Pharmacia). Partially purified hemolysin has HU(hemolytic unit).

Hemolytic activity was inhibited by about 99.9% until 1hr at 37℃ in the presence of ferric cation but not in the other cations such as Na^{+}, K^{+}, Li^{+}, Mg^{2+}, Mn^{2+}. These results were identical to the microscopic observation. Namely, sheep erythrocytes in the presence of ferric cations showed normal figures after treatment by hemolysin but disrupted in the other cations. While the protective action of ferric cation on sheep erythrocytes was decreased by adding 1mM EGTA. From these results, it can be suggested that ferric cation can rather protect erythrocytes and EGTA may act as a metal chelates. Further experiments are needed to determine mechanism of action of ferric cation to erythrocytes.

References

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