STUDIES ON THE PROTEOLYTIC ENZYME OF *CLOSTRIDIUM BOTULINUM*

Hirohide KODAMA

Department of Biochemistry,
Faculty of Veterinary Medicine,
Hokkaido University, Sapporo, Japan

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Earlier investigators have pointed out that a culture of *Clostridium botulinum* type A or B digests gelatin or some other native proteins. However, the characteristics of the proteolytic activity or biological role of it have remained uncertain.

In this paper, the author reports his investigation aimed first to detect and characterize proteolytic activity in culture supernatant of *Clostridium botulinum* type A (No. 38) or B (NIH). Simultaneously, culture supernatants of type E (11 strains) which are generally considered to be non or slightly proteolytic were also examined to detect proteolysis. Second, attempt was made to purify the proteolytic enzyme from type A (No. 38) culture supernatant. Finally, the actions of both the partially purified enzyme fraction and trypsin on type A (No. 38) or E (Iwanai) toxin were observed.

The results are summarized as follows:

1. The type A or B culture supernatant exhibited clear proteolytic activity showing pH optima at 7.0 requiring no activators. Normal sera, EDTA and copper sulfate inhibited the activity, whereas DFP or soybean trypsin inhibitor (typical inhibitor against trypsin) had no effect. On the other hand, the type E culture supernatants did not attack gelatin or casein under the conditions used. These observations show that the proteolytic action of the type A or B is different from that of trypsin though it may be classified as so-called trypsin type.

2. The specific activity of the proteolytic enzyme fraction which was partially purified from the type A culture supernatant with ammonium sulfate fractionation followed by acetone precipitation was approximately 80 to 85-fold as compared with that of the culture supernatant. Optimum pH and temperature of this fraction were 7.0 and 55°C respectively. The activity was also inhibited with normal sera, EDTA and copper sulfate thought the degree of inhibition was somewhat different from that of the culture supernatant. Polysaccharide which existed in this fraction could be separated by means of starch zone electrophoresis.

3. Toxicity found in the type A culture supernatant was not increased by the action of trypsin, while toxin obtained from the cells by sonic oscillation was

activated approximately 10-fold with trypsin, but not with the enzyme fraction. On the basis of these observations, the author considered that the proteolytic action of the type A culture may play an imoprtant role in the activation process of less active type A toxin though the enzyme itself has little lethal effect, and that the ability of trypsin to activate the type E toxin preparations is ascribable to lack or insufficiency of endogenous proteolytic enzyme in the culture.