# トリプシンによるヘモフィルス・パラガリナルムの赤血球凝集素の分離


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| 誌名 | 日本獣醫學雑誌 = The Japanese journal of veterinary science |
| ISSN | 00215295 |
| 著者 | 入谷, 好一 |
| 巻/号 | 41巻1号 |
| 掲載ページ | p. 69-71 |
| 発行年月 | 1979年2月 |

*Jap. J. vet. Sci.* **41**, 69~71 (1979)

BRIEF NOTE

# Separation with Trypsin of Hemagglutinin of *Haemophilus paragallinarum*

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(Received for publication May 24, 1978)

A considerable amount of research has been done on hemagglutinin (HA) of general bacteria [6, 7]. Kato et al. [5] first noted direct HA of *Haemophilus paragallinarum* (HG) 13 years ago, but did not describe the biological, biochemical or immunological properties of this HA in adequate detail. Recently, some biological and biochemical properties of HG-HA have been studied to clarify its characteristics [1, 2]. Those studies, however, were limited to crude HA in the presence of whole cells. Further studies showed that no active HA substances were released in the culture supernate of HG [3]. A method for separating HA from whole cells is needed in order to obtain further knowledge on the properties of HG-HA.

The present paper describes methods with trypsin for separating HG-HA from cells in an attempt to purify HA.

Kato's broth used for the propagation of 221 strain of HG was prepared as described previously [1]. Whole cells cultured in the broth at 37°C for 48 hours were collected by continuous-flow centrifugation at 10,000 rpm at 4°C at a flow rate of 4 *l*/hr. They were washed three times and resuspended in 2M NaCl solution. The packed cells were adjusted to about 200× the concentration of the initial culture broth and then stored in a refrigerator until use.

The packed cells were washed more than twice with phosphate-buffered saline (pH 7.0) (PBS) after incubation overnight at 37°C. The suspensions were added to an equal volume of neuraminidase (Sigma) dissolved at a concentration of 1 mg/m*l* in PBS, and incubated in a water bath at 37°C for 30 minutes. Next, neuraminidase was removed by washing twice with PBS. Then the cells were added to an equal volume of 0.25% crystal trypsin (PL-Biochemical Inc.) in PBS and incubated in a water bath at 37°C for 30 minutes. After centrifugation at 30,500×G for 30 minutes, the supernatant was decanted and mixed with half the volume of 0.25% trypsin inhibitor (Simga Type 11-0) in PBS. The resulting sediments were incubated for an additional treatment with trypsin.

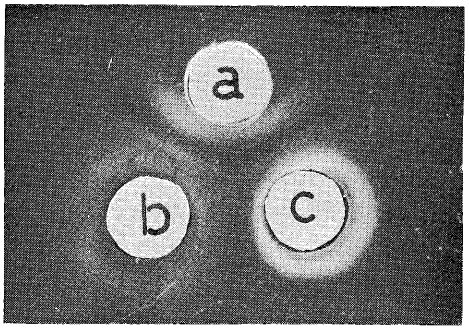
In another experiment, Nonident-P-40 (NP-40), Triton X-100 (TX-100), sodium deoxycholate (SDC), and sodium dodecyl sulfate (SDS) were used to separate HG-HA. Each detergent was added to the suspension of packed cells in PBS to a concentration of 1%. The mixture was stirred at room temperature for 15 to 20 minutes. The pellets and supernatant were removed

Table 1. Hemagglutination titer of *Haemophilus paragallinarum* after treatment with trypsin

| Process | | Experiment No. 1 | | 2 | | 3 | |
|---|---|---|---|---|---|---|---|
| | | Sed.* | Sup.** | Sed. | Sup. | Sed. | Sup. |
| Initial cells | | 1 : 128 | | 1 : 32 | | 1 : 64 | |
| After treatment with neuraminidase | | 1 : 256 | <1 : 1 | 1 : 16 | <1 : 1 | 1 : 64 | <1 : 1 |
| After trypsinization | First | 1 : 256 | 1 : 16 | 1 : 128 | 1 : 32 | 1 : 64 | 1 : 8 |
| | Second | 1 : 1042 | 1 : 8 | 1 : 128 | 1 : 8 | 1 : 256 | 1 : 8 |
| | Third | 1 : 256 | 1 : 8 | 1 : 128 | 1 : 4 | 1 : 128 | 1 : 1 |

Remarks.
* Sediment. ** Supernatant.
HA titer in the sediment was expressed for cells suspended in an equal volume of the supernatant.

Fig. 1. Agar-gel precipitation test of hemagglutinin of *Haemophilus paragallinarum* separated by trypsinization




Remarks.
a : Antigen of separated hemagglutinin after concentration by molecular filtration.
b : Antiserum to "a" antigen.
c : Antiserum to whole cell of *Haemophilus paragallinarum*.

from it by centrifugation at 30,500×G for 30 minutes and then dialyzed at 4°C by using nephlex or cellulose tubes.

Direct HA titer was determined by the methods described previously using a chicken red blood cell suspension [1].

Agar-gel precipitation (AGP) and hemagglutination-inhibition (HI) tests were performed by the methods described previously [4, 8]. Antiserum was obtained from chickens immunized with whole cells and separated HA solution.

As can be seen in Table 1, HA titers were found in the supernatant after trypsin treatment but not after treatment with neuraminidase alone. When HA activity was observed in the supernatant, HA titers in the sediment were enhanced by trypsin treatment. HA titers in the supernatant, however, were lower than those in the sediment, and decreased markedly by additional treatment with trypsin, as shown in Experiments 2 and 3.

HA titers in the sediment were enhanced by trypsin treatment, as in a previous experiment with crude trypsin (Difco) [1]. No reason for this enhancement is yet fully known.

On the other hand, the decrease in HA titer in the supernatant after the additional treatment with trypsin suggests that HG-HA may contain protein. Further studies are necessary to verify this suggestion.

When the supernatant obtained after trypsin treatment was subjected to the AGP test, at least two different precipitins were found in it (Fig. 1). The lines produced by homologous antiserum corresponded to those by antiserum to whole HG cells. Moreover, HI titers were found in the serum

of chickens immunized with the separated HA solution.

Sonicated antigen of whole cells of HG showed three precipitin lines in the AGP test [4, 10]. Furthermore, Sato and Shifrine [9] reported that a heat-labile antigenic factor of HG was extracted with phenol, although it was absent in boiled antigen. Recently, Iritani et al. [3] mentioned that extracellular antigen composed of at least two kinds of precipitin according to the AGP test existed in the culture supernate of HG, one precipitin being heat-resistant and the other not.

In this study, the HA solution separated by trypsin treatment contained at least two precipitins differentiated by the AGP test. Further studies are needed to determine whether the properties of trypsinized antigen are similar to those of extracellular antigen in the culture supernate and antigen boiled or extracted from whole cells.

When treated with NP-40, TX-100, SDC and SDS, HG cells were dissolved in these detergents and HA activity was destroyed.

Trypsinization could be used to separate HA from whole HG cells, even when the amount of HA was small. Experiments are now in progress to purify HA along this line. Such detergents as NP-40, TX-100, SDC and SDS, however, can not be used to separate HG-HA, as they destroy HA activity.

Acknowledgments: The author thanks Misses K. Tsuji and M. Mizuoka for their technical assistance.

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## 要　約

トリプシンによるヘモフィルス・パラガリナルムの赤血球凝集素の分離(短報)：入谷好一（塩野義製薬株式会社 油日ラボラトリーズ)——トリプシンと数種の界面活性剤とを使用し，ヘモフィルス・パラガリナルム(HG) の赤血球凝集素 (HA) の分離が行なわれた．ノイラミニデース前処理菌をトリプシン消化すると，他の菌体抗原を混存する HA が分離した．本 HA 液はニワトリに赤血球凝集抑制抗体を賦与した．しかし，界面活性剤は HA 性を失活させるため分離法として不適であった．トリプシン消化法の使用は HG-HA の性状解明に有用であると考えられた．