BRIEF NOTE

**Occurrence of Two Hemagglutinins on *Haemophilus*
paragallinarum Strain 221 and Comparison
of Their Properties**

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Some strains of *Haemophilus paragallinarum* (Hpg) agglutinate chicken red blood cells (RBC) [7], as well as any other *Haemophilus* species [1]. The authors [3-5] clarified the properties of hemagglutinin (HA) of Hpg involved. They also found out two differential HAs on Hpg cells. This paper deals with the occurrence of two HAs and their distinct nature.

Hpg strain 221 was cultured in chicken meat infusion broth at 37°C for 24 hr, and collected by continuous refrigerated centrifugation at 10,000 rpm at a flow rate of 4 l/hr. The collected cells were washed three times with phosphate buffer saline (PBS), pH 7.0, and suspended in PBS to prepare a suspension the density of which was 10 times as high as 0.4 with the absorbance at 540 m μ . This bacterial suspension was used as HA antigen termed type 1 HA. However, when the HA antigen was stored at 4°C for several months, or at room temperature for some weeks, it was sometimes inactivated naturally to lose its hemagglutinating activity to RBC of one-day-old chicks and partially susceptible adult chickens as previously described [6]. The HA antigen thus obtained was used as HA antigen termed type 2 HA.

Some differential properties of these two Hpg-HAs were determined by hemagglutinating activity to RBC of various animal species, including guinea pig, rat, mouse, rabbit, horse and sheep, and by the result of physicochemical treatment on the two HA antigens. These antigens were mixed with 0.25% trypsin, 0.1% chymotrypsin, 50 mM periodate sodium and 1% formaldehyde in PBS, respectively. The mixture was incubated at 37°C for 1 hr. After the enzymatic and chemical treatments terminated, the two HA antigens were washed three times with PBS and resuspended in the original volume of PBS. The effect of heat on them was tested at 65°C for 30 min or at 100°C for 10 min. The HA activities after these treatments were measured with RBC from susceptible and partially susceptible adult chickens free from specific pathogens at the authors' laboratories.

Table 1 shows a distinct agglutinability between the two HAs against chicken RBC. Type 1 HA agglutinated RBC from both chickens, but type 2 HA agglutinated only RBC from the susceptible chickens.

As shown in Table 2, type 1 HA could agglutinate RBC of various species, but type 2 HA could not agglutinate RBC of guinea

pig, horse or sheep. There was a clear difference between the two HAs in hemagglutinating activity on RBC of various

Table 1. Classification of two hemagglutinins (HAs) of *Haemophilus paragallinarum* by use of RBC of chickens of different susceptibility

Chicken group*	Chicken No.	HA titer	
		Type 1	Type 2
Susceptible	1	1:32	1:32
	2	1:64	1:32
	3	1:32	1:16
	4	1:16	1:16
Partially susceptible	1	1:32	<1:2
	2	1:16	<1:2
	3	1:64	<1:2
	4	1:32	<1:2

* Chickens from the LL flock at the authors' laboratories were used.

Table 2. Hemagglutination patterns of two HAs of *Haemophilus paragallinarum* against RBC of various animal species

Animal species	HA titer	
	Type 1	Type 2
Horse	1:16	<1:2
Sheep	1:32	<1:2
Guinea pig	1:16	<1:2
Rat	1:64	1:32
Mouse	1:64	1:32
Rabbit	1:32	1:32

species. This result shows that Hpg cells possess at least two HAs which must be distinguished from each other by using RBC of guinea pig, horse or sheep. In the hemagglutinating pattern against RBC of various species, *Escherichia coli* [2] and *Bordetella pertussis* [8] have been shown to possess some kind of HA.

The results of physicochemical treatment of the two HA antigens is shown in Table 3. It reveals that proteolytic enzyme inactivated type 1 HA activity on RBC of partially susceptible chickens, and converted phenotypically type 1 HA to type 2 HA, and that formaldehyde inactivated only type 1 HA. These results suggest that the surface component of type 1 HA may be protein moiety, and that proteolytic enzyme may cleave the protein. Consequently, type 2 HA was exposed. Type 1 HA must have masked type 2 HA. Since periodate sodium decreased type 1 HA activity on RBC of both groups of chickens, carbohydrate may have been concerned with type 1 HA activity. On the other hand, the component of type 2 HA was not clarified in this study. Both HAs were heat labile, and type 1 HA was more labile than type 2 HA.

In their previous paper [3], the authors

Table 3. Effect of physicochemical treatment on two HAs of *Haemophilus paragallinarum*

Treatment	HA titer			
	Type 1		Type 2	
	Positive*	Negative**	Positive	Negative
Trypsin (0.25%)	1:128	<1:2	1:32	<1:2
Chymotrypsin (0.1%)	1:32	<1:2	1:32	<1:2
Periodate (50 mM)	1:8	1:8	1:32	<1:2
Formaldehyde (1%)	<1:2	<1:2	1:32	<1:2
65°C for 30 min	<1:2	<1:2	1:16	<1:2
100°C for 10 min	<1:2	<1:2	<1:2	<1:2
Non-treatment	1:32	1:32	1:32	<1:2

* RBC from chickens susceptible to Hpg-HA.

** RBC from chickens partially susceptible to Hpg-HA.

reported the enhancement of hemagglutinating activity of Hpg-HA by trypsin. The results of the present investigation revealed that the enhancement was due to the appearance of a different type of Hpg-HA after trypsin treatment. It seems, therefore, that type 1 HA may exist in a more outer part of the cell surface than type 2 HA.

Taking these results into consideration, it is presumed that a natural conversion of type 1 HA into type 2 HA may be induced by hydrolysis of the cellular surface component responsible for type 1 HA activity during a long period of preservation.

No biological significance of the two HAs is known as yet. It is interesting to investigate the host-parasite relationship of the two HAs in infectious coryza of chickens.

In conclusion, cells of Hpg strain 221

possessed at least two HAs. There were differences in hemagglutinating activity on RBC of some animal species and in some physicochemical properties between the two HAs studied.

References

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要 約

ヘモフィルス・パラガリナルム 221 株の 2 種類の赤血球凝集素とその性状の比較 (短報): 山口 猛・入谷 好一 (塩野義製薬株式会社油日ラボラトリーズ)——ヘモフィルス・パラガリナルム 221 株において 2 種類の赤血球凝集素の存在が認められ、これをそれぞれタイプ 1HA およびタイプ 2HA と呼称した。タイプ 1HA は試験した全動物種の赤血球を凝集するが、タイプ 2HA は動物種によって異なった。タイプ 1HA の活性は蛋白分解酵素、ペリオデートおよびホルマリンにより影響を受けたが、タイプ 2HA は受けなかった。一方、両 HA 活性は易熱性であったが、タイプ 1HA の方がより熱感受性であった。