Hemagglutination by Pilus Antigen 987P of Enterotoxigenic Escherichia coli

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(Accepted for publication, August 17, 1987)

Abstract The hemagglutination (HA) by pilus antigen 987P of an enterotoxigenic Escherichia coli strain 987 was examined using fresh and glutaraldehyde (GA)-fixed erythrocytes (RBC) of various animals. Only when GA-fixed RBC was employed, a strain 987 exhibited striking HA activities. This was also demonstrated by using latex heads sensitized with the 987P antigen. The 987P-specific antiserum inhibited HA of strain 987 and 987P sensitized latex beads against GA-fixed RBC. We concluded that HA of strain 987 against GA-fixed RBC was specifically associated with the presence of 987P pilus antigen but do not exclude a possibility that adhesin is distinct from pili antigen.

Enterotoxigenic Escherichia coli (ETEC) cause an acute diarrhea both in humans and neonatal animals (3, 11). Enterotoxigenic colibacillosis involves the following sequence of events: colonization of the organism in the small intestine, and production of enterotoxin(s) which stimulates the small intestine to secrete body fluid. Colonization is an important step in ETEC infection, and attention has been paid to the pili of ETEC as a colonization factor. Many of them possess characteristics of attachment to the mucosa of the small intestine, as well as mannose sensitive or resistant hemagglutination (HA) which is relatively dependent on animal species of erythrocytes (RBC) (5). However, no HA of 987P against fresh RBC has yet been reported (6, 8, 9) except the description of Award-Masalmeh et al (1) who demonstrated a weak or questionable HA against fresh chicken RBC.

In this report, we examined HA of 987P using glutaraldehyde (GA)-fixed RBC, and described characteristics of this phenomenon.

ETEC strain 987 (09:K103:987P:NM) was used. Culture was in conformity to the method of Isaacson and Richter (6). Briefly, strain 987 was streaked onto blood agar plates and incubated at 37°C, and colonies containing cells in the piliated phase were identified by colonial morphology and by agglutination with 987P-specific antiserum. Strain 987 and 987P-specific antiserum were kindly supplied by Dr. R. E. Isaacson of Pfizer Central Research, Conn., U.S.A. Piliated (P+) and non-piliated (P−) cells selected by the above method were grown on trypticase soy broth (BBL). The purification of 987P was conducted according to the method of Isaacson and Richter (6). Sensitization of latex beads was conducted basically according
to the method of Evans et al (4). Latex beads with average size of 0.81 μm (Difco Laboratories, Detroit, Mich., U.S.A.) were washed with 0.1 M glycine buffer, pH 8.2, and resuspended in an original volume. The suspension was mixed with an equal volume of purified 987P (1.0 mg/ml). After incubation at 37 C for 3 hr, the sensitized beads were washed twice by centrifugation and resuspended in 0.05 M phosphate buffer (PB), pH 7.2, containing 0.1 % bovine serum albumin (BSA). The 987P-sensitized latex beads did not agglutinate with anti-O9:K57 and anti-O101:K103 sera.

Rabbit antisera specific for 987P, K88, and K99 were prepared by the method of Orskov et al (10) using strains 987, G-1253 (O147:K89) possessing K88ac, and EBK9-6 (O101:K+) possessing K99, respectively. Agglutinating titers of three pilus-specific sera were 1:512. Horse, bovine, pig, sheep, rabbit, guinea pig, and chicken RBC were used for HA assay. Freshly collected RBC were washed 3 times by centrifugation at 1,500 rpm for 15 min and were suspended in 0.5% in Dulbecco’s phosphate-buffered saline (PBS). The GA-fixed RBC, prepared by the method of Bing et al (2), were suspended in 1.0% in PBS and in PB containing 0.1% BSA.

HA test was carried out using macroplate method. P+ cells, P- cells, purified 987P, 987P-sensitized latex beads, and nonsensitized latex beads were used in this test. They were diluted twofold serially 1:1 to 1:4,096 in PBS (fresh RBC) or in PBS and PB containing BSA (GA-fixed RBC). The samples (0.4 ml) were mixed with an equal volume of fresh or GA-fixed RBC suspension, and the mixture was placed at 4 C and RT for 2 hr. The highest dilution showing complete HA reaction was taken as 1 HA unit. Mannose-resistant HA (MRHA) was detected by the addition of 1.0% D-mannose to each RBC suspension.

HA inhibition (HI) test was carried out according to the following method. To remove nonspecific reaction, antisera were absorbed by 4 volumes of 10% GA-fixed chicken RBC suspension. Twofold serial dilutions (0.2 ml) of 1:5 to 1:640 were made of absorbed sera with PB containing BSA. To each tube, an equal volume of hemagglutinin samples (4 HA units/0.2 ml) was added and left at RT for 30 min. Then, 0.4 ml of GA-fixed chicken RBC suspension was added. The HI titer was read at the highest serum dilution that inhibited HA completely after standing for 2 hr at RT.

Hemagglutinating pattern of strain 987 to various animal RBC is shown in Table 1. P- cells did not agglutinate fresh and GA-fixed RBC. Moreover, no fresh RBC was agglutinated by P+ cells. In marked contrast, when GA-fixed RBC was employed, P+ cells exhibited HA against these of various animals both at 4 C and RT. When PBS was used, the HA titer was <1:1 to 1:128, while it was 1:64 to 1:256 by PB.

MRHA by P+ cells, P- cells, purified 987P, 987P-sensitized latex beads, and nonsensitized latex beads with fresh and GA-fixed chicken RBC are shown in Table 2. P+ cells and 987P-sensitized latex beads caused MRHA, but P- cells, purified 987P, and nonsensitized latex beads did not.

HI tests with antisera prepared against 987P, K88, and K99 gave the following results. Specific antiserum for 987P inhibited HA reaction of P+ cells (1:160) and
of 987P-sensitized latex beads (1:80). But K88- and K99-specific antisera did not inhibit them (<1:5).

In this report, we examined hemagglutinating activity of 987P against fresh and GA-fixed RBC of various animals. Consequently 987P did not possess HA against any fresh RBC tested, but showed instantaneous and complete HA against GA-fixed RBC of various animals, especially GA-fixed chicken RBC. One possible explanation for this fact is considered as follows: the receptor for 987P-pili could exist within the inner side of the fresh RBC membrane, and could be exposed by GA treatment. But the mechanism is still unclear. It may be interesting to note that the HA titer was higher when PB rather than PBS was used. This result is probably due to the influence of hydrophobicity or ion electrode in buffer.

In HA test by various hemagglutinin samples, only P+ cells and 987P-sensitized latex beads showed HA against GA-fixed chicken RBC, but purified 987P did not.

Table 1. Hemagglutination of strain 987 (P+ cells) against fresh and glutaraldehyde (GA)-fixed erythrocytes (RBC) of various animals

<table>
<thead>
<tr>
<th>RBC</th>
<th>Temperature</th>
<th>Horse</th>
<th>Bovine</th>
<th>Pig</th>
<th>Sheep</th>
<th>Rabbit</th>
<th>Guinea pig</th>
<th>Chicken</th>
</tr>
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<tbody>
<tr>
<td>Fresh RBC</td>
<td>4C</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>in PBS</td>
<td>R.T.</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>GA-fixed RBC in PBS</td>
<td>4C</td>
<td>1:1</td>
<td>—</td>
<td>1:1</td>
<td>1:4</td>
<td>1:16</td>
<td>1:8</td>
<td>1:128</td>
</tr>
<tr>
<td>GA-fixed RBC in PB</td>
<td>4C</td>
<td>1:128</td>
<td>1:64</td>
<td>1:128</td>
<td>1:128</td>
<td>1:128</td>
<td>1:256</td>
<td>1:256</td>
</tr>
<tr>
<td>—</td>
<td>R.T.</td>
<td>1:64</td>
<td>1:64</td>
<td>1:64</td>
<td>1:64</td>
<td>1:64</td>
<td>1:128</td>
<td>1:256</td>
</tr>
</tbody>
</table>

Viable bacterial count: 1.0 x 10^10/ml.

a) Dulbecco's PBS.
b) 0.05 M PB, pH 7.2.
c) Room temperature.
da) <1:1.

d) Mannose-resistant hemagglutination.

Table 2. Hemagglutination of various treated 987P against fresh and glutaraldehyde (GA)-fixed chicken erythrocytes (RBC)

<table>
<thead>
<tr>
<th>RBC</th>
<th>Occurrence of HA</th>
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<tbody>
<tr>
<td></td>
<td>Whole cells 987P+</td>
</tr>
<tr>
<td>Fresh RBC</td>
<td>—</td>
</tr>
<tr>
<td>GA-fixed RBC</td>
<td>+</td>
</tr>
<tr>
<td>(MRHA^0)</td>
<td>(MRHA)</td>
</tr>
</tbody>
</table>

a) Whole cells (987+) and 987P sensitized-latex beads were prepared in 4 HA units.
b) Bacterial count; 1.0 x 10^10/ml.
c) Protein concentration; 1.0 mg/ml.
d) Latex beads of original concentration were used.
e) Mannose-resistant hemagglutination.
A similar finding has been reported in the case of CFA/I (4). In the HI test, 987P-specific antiserum inhibited the HA reaction due to P+ cells to an extent of 1:160 and that due to 987P-sensitized latex beads to an extent of 1:80; K88- or K99-specific antisera did not (<1:5).

These results suggested that HA of strain 987 against GA-fixed RBC was specifically associated with the presence of 987P pilus antigen but do not exclude a possibility that adhesin is distinct from pili antigen. However, it may be necessary to examine the presence of non-fimbrial MRHA such as afimbrial adhesins of pyelonephritic E. coli strains (7, 12) in the future.

We wish to thank Dr. R.E. Isaacson, Pfizer Central Research, Connecticut, U.S.A., for kindly supplying E. coli strain 987 and 987P-specific antiserum, and Dr. A. Ghoda, Kitasato University, Shirokane, Minato, Japan, for reviewing the manuscript.

REFERENCES


(Received for publication, March 23, 1987)