Immunological Cross-Reactivity of the Fragments of Staphylococcal Enterotoxins A and E Generated by Digestion of Proteolytic Enzymes

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ABSTRACT. Three major fragments were generated by limited digestion of staphylococcal enterotoxins A (SEA) and E (SEE) with papain, whereas five major fragments were generated by limited digestion with staphylococcal protease V8 (V8). All of these fragments were detected by immunoblotting with polyclonal anti-SEA and -SEE sera. Some of generated fragments were detected by monoclonal antibodies (MABs) with specificities for SEA (A-111 and A-211), SEE (E-142), or both (AE-32, AE-37, and AE-53). This indicates that fragments of SEA and SEE containing the type-specific and cross-reacting epitopes may be generated by digestion of the toxins with either papain or V8.—KEY WORDS: cross-reactivity, enterotoxin A, enterotoxin E, proteolytic fragment, S. aureus.


The staphylococcal enterotoxins (SEs) induce emesis and diarrhea in humans and other primates [1, 2]. They are serologically classified into at least five types: A (SEA), B (SEB), C (SEC1, SEC2, and SEC3), D (SED), and E (SEE). Although SEs are serologically distinct, cross-reacting antibodies in polyclonal sera to purified SEs have been observed, particularly SEB and SEC1 [10, 17, 18, 21] or SEA and SEE [3, 10, 14]. Specific and cross-reacting epitopes on SEB and SEC1 have been studied using tryptic fragments and different monoclonal antibodies (MABs) [21]. Because limited trypsin digestion of SEB yields two polypeptide chains [19], whereas that of SEC1 generates three peptides [17]. Specific and cross-reacting antigens of SEA and SEE have not been studied because SEA is resistant to trypsin and a-chymotrypsin [6, 19]. However, it has been reported that SEA is susceptible to papain, pepsin, pronase [6], and staphylococcal protease V8 (V8) [20] digestion. Limited digestion of SEA with papain generated three major and one minor fragments [6], whereas that of SEA with V8 yielded 6 fragments [20]. Many peptides with molecular weights of lower than 10,000 have been generated by digestion of SEA with pepsin and pronase because partially denatured SEA was susceptible to these enzymes [6]. Although MABs to SEA and SEE have been produced [4, 5, 12], attempts to study the cross-reacting epitopes between SEA and SEE have not been made. The present study was initiated to generate fragments of SEA and SEE by cleavage with papain and V8 and to localize the epitope on the generated fragments by use of different MABs specific for SEA, SEE, or both recently prepared in this laboratory.

MATERIALS AND METHODS

Purification of SEs: Purification of SEA and SEE were carried out by methods described previously [13, 14]. The purity of these SEs was determined by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) [9]. Specific antiserum against purified SEA and SEE were prepared in rabbits [13, 14].

Immunization and hybridization: Adult Balb/c mice (8 weeks old) were immunized by intraperitoneal injection of 30 μg of purified SEA or SEE emulsified in Freund’s complete adjuvant (Difco Lab., Detroit, Mich. U.S.A.) at 0, 2, and 4 weeks. Six to seven weeks later they were given an intravenous injection of 50 μg of purified SEA or SEE without adjuvant. Spleen cells were removed 4 days after the booster injection and fused with myeloma cells SP2/0 Ag14 maintained in Dulbecco’s modified Eagle’s medium (Gibco Lab., New York U.S.A.) supplemented with 10% fetal calf serum (Flow Lab. Inc., U.S.A.) by use of 50% polyethylene glycol 4000 (Wako Pure Chem. Indst. Ltd., Osaka, Japan) according to the methods described previously [7, 8]. Hybrid cells were selected in HAT medium and hybridomas producing antibodies were cloned by the limiting dilution method. The stable hybridomas obtained are pre-
Table 1. Reactivities of murine monoclonal antibodies (MAb) against staphylococcal enterotoxins A (SEA) and E (SEE)

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<th>Immunogen</th>
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<tr>
<td>SEA</td>
<td>A-111 (IgG₁)</td>
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<td>A-211 (IgG₂)</td>
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<td>SEE</td>
<td>E-142 (IgG₁)</td>
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Reactivities of MAb to SEA and SEE were determined by enzyme-linked immunosorbent assay (ELISA) according to the methods described previously [15] and the Western blotting [22].

Limited digestion of SEs with proteolytic enzymes for fragmentation: For treatment of SEA and SEE with proteolytic enzymes, 5 mM phosphate buffer, pH 6.5 and 0.125 M Tris-HCl, pH 7.8, containing 0.5% SDS and 10% glycerol were used for digestion of the toxins with papain and V8, respectively. For proteolytic treatment, toxin-enzyme ratio was 10:1. For papain digestion, 5 µl of SEA (or SEE) (10 mg/ml) was mixed with 5 µl of papain (1 mg/ml). For V8 digestion, 10 µl of SEA (or SEE) (10 mg/ml) was mixed with 20 µl of the SDS sample buffer [9] and the mixture was boiled for 1 min. After boiling, 64 µl of V8 (156 µg/ml) was added to the mixture. Limited digestion was carried out with papain at 37°C for 3 hr and with V8 for 5 min, respectively. Before the reaction was stopped by diluting the digestion mixture into 40 µl of the SDS sample buffer [9] and boiling for 1 min. The fragments obtained were separated by SDS-PAGE under reducing condition [9] using 15% gel for the papain digest and 12.5% gel for the V8 digest, respectively. Peptides transferred from gels were detected by each MAb and the homologous polyclonal rabbit antiserum using 4-chloronaphthol (Bio-Rad Lab. Richmond, CA., U.S.A.) [22]. The molecular weights of the generated fragments were calculated by SDS-PAGE [23] using electrophoresis calibration kit (Pharmacia, Uppsala, Sweden).

Results and discussion

The molecular weight of undigested SEA was calculated to be 29,000 by SDS-PAGE (Fig. 1). In addition to undigested SEA, three major fragments (designated I, II, and III) were generated by papain digestion of SEA. Their molecular weights were calculated to be 18,000 for I, 14,000 for II, and 12,500 for III, respectively (Fig. 1). Similar results were obtained with the papain digest of SEA with generation of three major peptides (MW: 17,500, 16,200, and 14,000) and one minor peptide (MW: 15,000) [6]. All of the fragments (I, II, and III) were found to be reactive with polyclonal anti-SEA antibody (Fig. 1). MAb A-111 and AE-53 reacted with only fragment I, whereas MAbs A-211, AE-32, and AE-37 reacted with fragments I and II (Fig. 1). Thus, one can conclude that the specificities of the latter three MAbs are different from the first two MAbs. None of the MAbs reacted with fragment III. It is apparent that the epitope(s) on fragment III was less reactive because no MAbs reacting with this
fragment were isolated.

Five fragments (I, II, III, IV, and V) were obtained by digestion of SEA with V8 (Fig. 2): similar results with this enzyme were obtained by other investigators [20]. The molecular weights of these fragments were calculated to be 26,000 for I, 25,000 for II, 24,000 for III, 22,500 for IV, and 22,000 for V, respectively. However, these fragments were further digested to peptides with molecular weights of lower than 10,000 when treatment was continued for longer than 5 min at 37°C. It is apparent that small peptides were successively removed as the digestion continued, reducing the original molecule to the 22,000 molecular weight. It is not known whether small peptides were removed from only one end of the molecule, such as the C-terminal end, or from the N-terminal end as well. In any case, polyclonal anti-SEA as well as MAbs AE-32, AE-37, and AE-53 reacted with the 22,000 dalton molecule. However, at least one peptide was removed or changed by the digestion because MAbs A-111 and A-211 (Fig. 2) no longer reacted with the 22,000 dalton peptide. One may conclude from these results that the epitope(s) specific for SEA are located on the part of the molecule removed by the digestion, whereas the cross-reacting epitope(s) are located on the 22,000 dalton peptide. Peptides with molecular weight of less than 10,000 dalton did not react with any of the antibodies, either polyclonal antibody or MAbs.

By digestion of SEE with papain and V8, similar results were also obtained in SDS-PAGE (Figs. 3 and 4). The molecular weight of undigested SEE was calculated to be 30,000 by SDS-PAGE. Three major fragments were generated by papain digestion of SEE: these fragments were also designated I, II, and III (Fig. 3). Their molecular weights were calculated to be 15,500 for I, 14,000 for II, and 11,000 for III, respectively. All these fragments were detected by polyclonal anti-SEE serum. MAbs AE-37 and E-142 reacted with fragment I, whereas MAbs AE-32 and AE-53 did not react with any fragments (Fig. 3). It is apparent that an epitope on fragment I may be specific for SEE because MAb
Fig. 4. Western blots of V8-digested staphylococcal enterotoxin E (SEE) exposed to polyclonal and monoclonal antibodies. Staining of untreated (lane 1) and V8-digested (lane 2) SEE with Commassie brilliant blue. Western blots of V8-digested SEE with polyclonal anti-SEE antibody (lane 3), E-142 (lane 4), AE-32 (lane 5), AE-37 (lane 6), and AE-53 (lane 7).

E-142, reacting with SEE, reacted with this fragment. A second epitope on this fragment reacted with MAb AE-37. The epitope(s) recognized by MAbs AE-32 and AE-53 is different from the one reacting with MAbs AE-37 and E-142 although MAbs AE-32 and AE-37 are reactive with both SEA and SEE (Table 1). These findings are supported by the SDS-PAGE results with the V8 digest of SEE (Fig. 4). The V8 digest of SEE generated 5 fragments (I, II, III, IV, and V) (Fig. 4), with their molecular weights calculated to be 26,000, for I, 25,000 for II, 23,000 for III, 21,000 for IV, and 19,000 for V, respectively. All of these fragments reacted with polyclonal anti-SEE serum and MAb AE-37, whereas fragments I, II, and III reacted with MAb E-142, indicating that the epitope(s) specific for SEE may be located on fragment III. Fragments IV and V were detected by MAb AE-37, indicating that the cross-reacting epitope(s) between SEA and SEE may be located on these fragments. The epitope with which MAb AE-37 reacted apparently is different from the epitopes reacting with MAbs AE-32 and AE-53. As was the case with V8 digestion of SEA, treatment of SEE with V8 for longer than 30 min at 37°C generated fragments less than 10,000 daltons. These were unreactive with any of the antibodies used.

As shown here, 3–5 fragments can be generated by limited digestion of SEA and SEE with papain and V8. The isolated fragments should be useful in identifying the type-specific, cross-reacting epitope(s), or both. Further studies in this area should provide important information in this regard.

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