Note

Construction of a LukS-PV Mutant of a Staphylococcal Panton-Valentine Leukocidin Component having a High LukS-like Function

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A 2-residue (D11P13) segment of LukS of a staphylococcal leukocidin component is an essential region for the hemolytic function of LukS towards rabbit erythrocytes in the presence of LukF. Here, we report that insertion of D, I, or AA residue(s) between A11 and E12 residues of LukS-PV, in which the 2-residue D11P13 segment in LukS was absent, conferred the full LukS function on LukS-PV, which has only 4% hemolytic activity of that of LukS towards rabbit erythrocytes.

Key words: staphylococcal leukocidin; Panton-Valentine leukocidin; LukF-PV; LukS-PV

Staphylococcus aureus produces Panton-Valentine leukocidin (PVL) and leukocidin (Luk), which function as two component toxins in the disruption and lysis of the target cells. PVL and Luk consist of LukF-PV and LukS-PV, and LukF and LukS, respectively. The study of the cytolytic activity of combinations of the components showed that LukF and LukS cooperatively and strongly lyse rabbit erythrocytes besides human and rabbit PMNLs. In contrast, the combination of LukF and LukS-PV has only 4% hemolytic activity of that in combination [LukF-LukS]. We showed that the absence of a 2-residue (DI) segment, which was identified to be an essential region for the hemolytic function of LukS, between A11 and E12 residues in LukS-PV causes a drastic descent of the hemolytic activity, and that the insertion of the 2-residue segment between A11 and E12 residues of LukS-PV conferred the full hemolytic function on LukS-PV. This prompted us to investigate an absolute requirement of the 2-residue segment. Here, we assayed the leukocytolytic and hemolytic activities of mutant proteins of LukS-PV or LukS, which were expressed in E. coli cells harboring the appropriate plasmid containing the additional or truncated structural gene of lukS-PV or lukS, respectively, and showed that insertion of either D, I, or AA residue(s) between A11 and E12 of LukS-PV confers the hemolytic activity on LukS-PV towards rabbit erythrocytes.

We started by constructing plasmids containing a series of the mutant lukS-PV or lukS genes using overlapping extension method. In each case, the gene to be expressed was cloned into the plasmid pTrc99A (Pharmacia, Uppsala, Sweden) to have a high level of IPTG-inducible expression in E. coli DH5a. The recombinant plasmids were constructed by the method described in the footnote of Table. As a result of these manipulations, 7 different plasmids that contained the mutants of lukS-PV or lukS corresponding to the residue(s) inserted and deleted between A11 and E12 of LukS-PV and between S11 and E14 of LukS, respectively, were constructed. The plasmids were designated as pMPVS8, pMPVS9, pMPVS10, pMPVS11, pMLS2, pMLS3, and pMLS4. The mutant proteins except MLS3 were expressed in E. coli harboring the appropriate plasmid and prepared from the sonicated extract of the cells from one liter of culture. The plasmid pMLS3, which corresponds to the deletion mutant of L10 of LukS, failed to be expressed in E. coli. The sonicated extracts were put on a hydroxylapatite column (0.5 × 5 cm) equipped with 80 mM potassium phosphate buffer, pH 6.8 (buffer A) separately. After the column was washed with buffer A, each mutant protein was eluted from the column with 0.8 M potassium phosphate buffer, pH 6.8, and the eluate was dialyzed against buffer A. The dialyze was put on a TSK gel SP-5PW column (Tosoh, Tokyo), using a linear gradient NaCl (0–0.8 M) in buffer A. MPVS8, MPVS9, MPVS10, and MPVS11 proteins were eluted with 0.11, 0.12, 0.09, and 0.47 M NaCl, respectively, as a symmetrical peak of the protein. On the other hand, MLS2 and MLS4 mutants of LukS were eluted with 0.43 and 0.42 M NaCl, respectively, as a symmetrical peak of protein. All mutant protein preparations, MPVS8 (2.6 mg), MPVS9 (4.1 mg), MPVS10 (1.5 mg), MPVS11 (2.7 mg), MLS2 (8.1 mg), and MLS4 (1.5 mg) were electrophoretically homogeneous (data not shown).

When leukocytolytic and hemolytic activities of the LukS-PV and LukS mutants shown in Table were measured in the presence of LukF, the following findings became evident: (I) All mutant proteins as well as intact LukS-PV and LukS had full leukocytolytic activity on human PMNLs (Table). It is reasonable that they have the leukocytolytic activity, because they have the essential segments (R23RRT244 for LukS-PV and K243RST246 for LukS) in their C-terminal regions, which are phosphorylated by protein kinase A on human leukocytes for the Luk function.‡ (II) Mutant proteins MPVS8

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Abbreviations: LukS, LukS component of leukocidin; LukF, LukF component of leukocidin/γ-hemolysin; PMNLs, polymorphonuclear leukocytes

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and MPVS9 with D or I residue inserted between A11 and E12 of LukS-PV, respectively, had hemolytic activity to the same or higher extent than that of LukS (Table, lanes 3 and 4). (III) The mutant protein MPVS10, in which I and D residues were inserted in order between A11 and E12 of LukS-PV had also showed approximately 3 times higher hemolytic activity than that of the mutant protein MPVS6, which was characterized previously, and in which both D and I residues were inserted in order between A11 and E12 of LukS-PV (Table, lanes 5 and 2). (IV) MLS2 which is a D3-deletion mutant of LukS had hemolytic activity to the same or higher extent to that of the combination [LukF-LukS] (Table, lane 9). (V) The LukS mutant, MLS4, in which 2-residue [D12I13] segment was replaced by I12D13, showed 2 times higher hemolytic activity than that of LukS (Table, lane 10). (VI) However, both intact LukS-PV and LukS mutant without the 2-residue segment (MLS1) have only 4 and 12% hemolytic activity of that of the intact LukS, respectively (Table, lanes 1 and 8). These data demonstrated that the insertion of either a D or an I residue between A11 and E12 of LukS-PV confers the hemolytic activity to the same or a higher extent to that of LukS on itself, and also suggested the possible insertion of a different amino acid(s) residue from D and I between A11 and E12 of LukS-PV for conferring hemolytic activity on itself. Accordingly, a mutant protein MPVS11, in which a 2-residue (AA) segment was inserted between A11 and E12 of LukS-PV was constructed using primers described in the Table. MPVS11 was expressed in E. coli DH5α, purified by the method described above, and assayed for the toxin activities towards human PMNLs and rabbit erythrocytes in the presence of LukF. The mutant protein showed both toxin activities to the same or a higher extent to that of LukS (Table, lane 6). Thus, we concluded that the insertion of D, I, or AA between A11 and E12 of LukS-PV confers the hemolytic activity on LukS-PV towards rabbit erythrocytes.

Recently, we reported that LukF and LukS assemble into a ring-shaped approximately 200-kDa complex on the rabbit erythrocytes as well as human PMNLs in a molar ratio of 1:1, which forms a membrane pore with a functional diameter of approximately 2.0 nm. Using our established systems, we examined the binding activity of LukS-PV, LukS, and their mutants to LukF on the rabbit erythrocytes. The mutants that had hemolytic activities as well as the intact LukS had full binding activity to LukF to form the approximately 200 kDa complex on the rabbit erythrocytes. However, no LukF-LukS-PV complex was visible (data not shown). The data indicate that the presence of either D12 or I12 residue of LukS, and the insertion of D, I, or AA between A11 and E12 of LukS-PV also play a pivotal role in the binding of S component and LukF to form the membrane pore on the rabbit erythrocytes. Recently, Gouaux et al. reported that from the analysis of the aligned sequence of the staphyloccocal α-hemolysin (Hla) that assembles from a water-soluble, monomeric species to a membrane-bound heptamer on the surface of target cells, the proto- meric structures of Hla, PVL, and Luk components are quite similar. Eventually, the X-ray analysis of the crystal structure of membrane-bound form of Hla and the water-soluble form of LukF found to be identical except only for the stem domain of each component.
basis of the data, the DI segment of LukS might be located between the amino latch and three-strand $\beta$-sheet domains, which forms a “cap structure” in the complex comprising of LukF and LukS, as a linker and might play an important role on the formation of the complexes of LukS with LukF on the rabbit erythrocytes.

Although the reason why the insertion of D residue or ID segment between A$^{11}$ and E$^{12}$ of LukS-PV caused higher hemolytic activity than that of I, DI, or AA segment is not clear yet, it is likely that the sequence of D$^{12}$ and E$^{13}$ in order has a positive effect on the hemolytic activity of the LukS-PV mutants.

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