Figures and figure supplements

An internal thioester in a pathogen surface protein mediates covalent host binding

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Figure 1. Domain architectures of TED-containing proteins of clinically relevant bacteria. C, T and Q indicate positions of conserved motifs. The MEME signatures (Bailey et al., 2009) derived from 54 sequences (Figure 1—figure supplement 1) are also shown with the thioester-forming residues in red; proline, hydrophobic, polar and small residues in yellow, green, blue and grey, respectively. TIE, thioester, isopeptide, ester domain protein; TEP, thioester domain containing protein.

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**Figure 1—figure supplement 1.** Sequence alignment of 54 TEDs from 40 species. Proteins with names shown in red were investigated in this study. Blue and green Uniprot accession codes belong to proteins of firmicutes and...
actinobacteria, respectively. Secondary structure elements of SfbI-A40-TED are displayed on top. The thioester-forming Cys and Gln residues are highlighted in red, partially conserved residues are shaded grey (threshold for shading 85%). The following proteins are included in the alignment. UniProt accession codes and species names are given, also protein names in parentheses, where available: Q1JDZ6 (SfbI-A40), *Streptococcus pyogenes*; Q711F8 (SfbI-A346), *S. pyogenes*; Q01924 (SfbI-A20), *S. pyogenes*; Q6EWI8 (GfbA), *Streptococcus dysgalactiae* subsp. *equisimilis*; Q8G9G1 (FbaB), *S. pyogenes*; Q8GRA2 (Cpa-TED2), *S. pyogenes*; B1R775 (CpTIE), *Clostridium perfringens*; H2GB86 (CodTIE), *Corynebacterium diphtheriae*; A5MCJ6/Q4K1R7 (PnTIE), *Streptococcus pneumoniae*; C3DPD7 (BaTIE), *Bacillus anthracis*; I3GYQ5/I3HJ59/I3HKL2 (SaTIE), *Staphylococcus aureus* VRS11b; Q17Z20 (CdTEP), *Peptoclostridium difficile*; F22X80, *Gemella haemolysans*; U2Q1B9, *Gemella bergeriae*; E1JLX8, *Lactococcus salivarius*; P72416 (FnZ), *Streptococcus equi*; Q0MANS (FneC), *Streptococcus equi* subsp. *equi*; T1ZSL1, *Streptococcus constellatus*; D0UF33 (Sbs13), *Streptococcus gaiolyticus*; G5J2C9, *Streptococcus ictaluri*; A8SJ15, *Parvimonas micra*; G4D2J9, *Peptoniphilus indolicus*; D3MRS9, *Peptostreptococcus anaeobius*; K0ZKB8, *Actinomyces turicensis*; D7BLE5, *Arcanobacterium haemolyticum*; H2H5E2, *C. diphtheriae*; H2GKP8, *C. diphtheriae*; D9QDT9, *Corynebacterium pseudotuberculosis*; G0CMN3, *Corynebacterium ulcerans*; E6EBZ4, *Propionibacterium acnes*; D2Q6N9, *Bifidobacterium dentium*; S4GNG3, *Gardnerella vaginalis*; D6L3Z6, *Parascardovia denticolens*; J0D4V1, *Scardovia wiggsiae*; B5UW14, *Bacillus cereus*; C3AWK0, *Bacillus mycoides*; E0WKP3, *Listeria grayi*; Q8Y804, *Listeria monocytogenes*; H7CR09, *L. monocytogenes*; W7CRB3, *Listeria weihenstephanensis*; Q833P7, *Enterococcus faecalis*; R2PL19, *Enterococcus faecium*; S4EXK4, *E. faecium*; T4YK58, *P. difficile*; X4QXD7, *Trueperella pyogenes*; D4YNAG, *Brevibacterium mcbrellneri*; D7AUS9, *Nocardiopsis dassonvillei*.

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Figure 1—figure supplement 2. 10 of the 12 purified TEDs used in this study. SDS-PAGE showing the final purity of 10 of the 12 TED samples. The second bands observed for CodTIE and BaTIE likely represent breakdown products of the TEDs.

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Figure 1—figure supplement 3. Identification of the thioester-forming Gln residues in class-II TEDs. MS of tryptic digests of TEDs following incubation with methylamine and blocking of the resulting free Cys thiol with iodoacetamide (IAA). The thioester-forming Gln residues appear as methylated Gln (Q(Me)). Precursor signals matching the methylated tryptic fragment are shown. The masses do not match any other tryptic fragments of the TEDs. $\Delta$, difference between experimental and theoretical mass. The BaTIE-TED fragment contains two Gln residues, and MS/MS analysis was used to unambiguously identify Gln266 as the residue involved in thioester formation.

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Figure 2. Crystal structures of TEDs. (A) Cartoon representations of crystal structures of SfbI-A40-TED (blue), CpTIE-TED (burgundy) and PnTIE-TED (yellow) with thioesters shown as sticks. Variable loops adjacent to the thioester are colored red, purple and green, respectively. (B) Close-up views of the thioesters. Residues forming the thioesters are shown overlaid with electron density (2mF\textsubscript{obs} – DF\textsubscript{calc} contoured at 1.0\(\sigma\)). The ‘Q’, ‘W’ and ‘ζ’ residues of the TQxxΦWΦx\(\zeta\) motif are also shown. (C) Surface representations with variable loops colored as in A.

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Figure 2—figure supplement 1. Comparison of TED structures. (A) Overlay of SfbI-A40-TED (blue), CptTIE-TED (burgundy), Pn-TIE-TED (yellow), Cpa-TED2 (pale blue, PDB:2XI9) and Cpa-TED1 (grey, PDB:4C0Z). Thioester and TQxxΦWΦxζ motif residues are shown as sticks. (B) Crystal structure of CptTIE-TED:Cys138Ala with adjacent variable loop colored cyan. (C) Overlay of CptTIE-TED (burgundy) and CptTIE-TED-Cys138Ala (orange).

DOI: 10.7554/eLife.06638.009
**Figure 3** SDS-PAGE analysis of TED-fibrinogen (Fg) adduct formation. (A) Stable adducts between fibrinogen and TEDs revealed by SDS-PAGE. Red asterisks, adduct bands. (B) Cys-to-Ala mutants of TEDs do not result in adduct formation after incubation with Fg. (C) Schematic representation of the intermolecular isopeptide bond formed between a Lys residue of fibrinogen Aα and the Gln residue of the TED.

DOI: 10.7554/eLife.06638.012
Figure 3—figure supplement 1. Effect of acetylation of Lys side chains on fibrinogen (Fg):TED adduct formation. The hash symbol indicates protein pre-incubated with sulfo-NHS acetate. Red asterisks highlight the positions of adduct bands. DOI: 10.7554/eLife.06638.013

Figure 4. TED-fibrinogen (Fg) adduct formation in plasma pull down assays. (A) Schematic representation of the isopeptide domain (green) complementation used in the pull-down assays. Pink triangle, tobacco etch virus protease cleavage site. (B) Sfbl and FbaB-TEDs pull down fibrinogen from blood plasma, forming covalent adducts (red asterisks). ‘a’ denotes bands corresponding to human serum albumin. Bands below 31 kDa are breakdown products of tagged TEDs. DOI: 10.7554/eLife.06638.014
Figure 4—figure supplement 1. TED:fibrinogen (Fg) adduct formation in plasma pull down assays. An overloaded gel of the plasma pull-down better shows adduct formation between fibrinogen and TEDs, and its dependence on the thioester. P = plasma depleted of the majority of albumin by blue sepharose chromatography. Red asterisks, adduct bands, ’a’ and ’d’ denote human serum albumin and Fg γ dimer, respectively.
DOI: 10.7554/eLife.06638.015
Figure 5. Mass spectrometric identification of the SfbI-TED and FbaB-TED target residue in fibrinogen (Fg). (A) Precursor ion masses identified in tryptic-digest nanoLC-MS² of excised adduct gel bands for different samples. (B) Several charged states are observed in nanoLC-MS² of cross-linked precursor peptides obtained by tryptic digestion of adducts formed by TEDs with fibrinogen or in plasma pull-downs. (C) Fragmentation nanoLC-MS² spectra of the cross-linked precursor obtained for SfbI-A40-TED in a plasma pull-down. The low and high mass range spectrum is shown on the left and right, respectively. Fragmentations observed in nanoLC-MS² are indicated in the schematic drawings above the spectra by hooks, y-series on top of sequences, b-series below sequences. Numbers correspond to positions in the cross-linked fragments; red, SfbI-A40-TED; blue, fibrinogen Aα.

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Acetylation of Lys residues in the fibrinogen \( \alpha \) subunit in absence and presence of SfbI-A40-TED. Sequence coverage by peptide mass fingerprinting is indicated by colors mapped onto the sequence of human fibrinogen subunit \( \alpha \). Grey, signal sequence (not present in mature fibrinogen \( \alpha \)); red, trypsin coverage; blue and green, additional coverage obtained by endoproteinase GluC and chymotrypsin digestion, respectively; black, no coverage. All Lys residues are highlighted in boldface. Underlined K indicates that the residue was observed in its acetylated form; non-underlined K were observed only in the non-acetylated form.

**Figure 5—figure supplement 1.**

**Adduct band, fibrinogen + SfbI-A40-TED**

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<thead>
<tr>
<th>Sequence</th>
<th>Acetylation</th>
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<tr>
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**Figure 5—figure supplement 2.** Structure of fibrinogen with important sites labeled. The positions of Lys100 (magenta) and RGDF platelet-binding motif (cyan) are highlighted on the crystal structure of Fg (PDB:3GHG). \( \alpha \), \( \beta \) and \( \gamma \) chains are colored blue, green and red, respectively.

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Figure 6. Thioester-dependent binding of bacteria to fibrin. (A) and (B) fibrin binding by *L. lactis* expressing SfbI-A40. (C) SfbI-A40:Cys109Ala does not confer fibrin-binding activity to *L. lactis*. (D) Control of *L. lactis* transformed with empty pOri23 plasmid. All scale bars 2 μm, except in the insert in panel B, 0.2 μm. DOI: 10.7554/eLife.06638.019

Figure 6—figure supplement 1. Immunogold labeling of SfbI-A40 and SfbI-A40:Cys109Ala on *L. lactis* surfaces. A and B *L. lactis* expressing SfbI-A40. (C) *L. lactis* expressing SfbI-A40:Cys109Ala. (D) *L. lactis* transformed with empty pOri23 plasmid shows essentially no cross-reactivity of the antibody with the bacterial surface. All scale bars 0.2 μm. DOI: 10.7554/eLife.06638.020
**Figure 6—figure supplement 2.** Fibrin clot binding of *L. lactis* expressing SfbI variants. A control of *L. lactis* transformed with empty pOri23 plasmid is also shown. All scale bars 10 μm except in the right column, 2 μm. White arrows point to bacteria in the experiments with the Cys-to-Ala mutants and the empty plasmid.

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Figure 7. Thioester-dependent binding of SfbI-A40-TED to A549 cells. (A) Schematic representation of the covalent cell labeling experiment. After incubation with cells, isopep-tag (iPT) labeled TED is detected by GFP fused to siPD. (B) SfbI-A40-TED and SfbI-A40-TED:Cys109Ala binding to A549 cells before and after incubation with interleukin-6.

Figure 7. continued on next page
(IL6) and dexamethasone (dex). No TED, siPD-GFP controls. Cell nuclei appear in blue. Scale bars, 200 μm. (C) Western blot of whole-cell extracts of induced and non-induced A549 cells, probed with an anti-fibrinogen α antibody. The same membrane was re-probed with an anti-β-actin antibody (shown in separate panel). MW, molecular weight markers; TED, cells incubated with SfbI-A40-TED-iPT; TED C-A, cells incubated with SfbI-A40-TED: Cys109Ala-iPT.

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