SUPPLEMENTAL MATERIAL

THE STRUCTURAL BASIS FOR INHIBITION OF THE CLASSICAL AND LECTIN COMPLEMENT PATHWAYS BY *S. AUREUS* EXTRACELLULAR ADHERENCE PROTEIN

Jordan L. Woehl, Kasra X. Ramyar, Benjamin B. Katz,

John K. Walker, and Brian V. Geisbrecht

This file contains one supplemental table, four supplemental figures,

and their corresponding legends

Table S1. Supporting Table of IC_{50} Values and Statistical Analyses for Inhibition of Lectin Pathway Dependent Deposition of C3b and C5b-9 by Various Eap Proteins.

Protein	C3b Deposition by Lectin Pathway			C5b-9 Deposition by Lectin Pathway		
	IC_{50}[1]	Confidence Interval (95%)	R^2	IC_{50}	Confidence Interval (95%)	R^2
EapFL	71.1 nM	60.5 to 83.5 nM	0.985	74.9 nM	69.5 to 80.8 nM	0.987
Eap12	11.7 μM	7.53 to 18.0 μM	0.967	12.3 μM	6.41 to 23.4 μM	0.968
Eap23	5.68 μM	3.49 to 9.25 μM	0.991	3.77 μM	3.37 to 4.21 μM	0.994
Eap34	227 nM	202 to 255 nM	0.995	163 nM	151 to 176 nM	0.995
Eap1	27.3 μM	24.2 to 30.9 μM	0.992	16.1 μM	11.9 to 21.8 μM	0.971
Eap2	108 μM	36.2 to 321 μM	0.969	113 μM	27.8 to 461 μM	0.960
Eap3	14.9 μM	13.8 to 16.3 μM	0.975	12.8 μM	10.1 to 16.2 μM	0.988
Eap4	11.8 μM	9.85 to 14.2 μM	0.979	5.44 μM	4.85 to 6.09 μM	0.988
Eap34-KΔA	984 nM	819 to 1180 nM	0.983	n/d[2]	n/d	n/d
Eap34-KΔE	2.24 μM	1.93 to 2.61 μM	0.973	n/d	n/d	n/d

[1]All statistical parameters were determined after curve fitting by GraphPad Prism 6.0.

[2]n/d signifies that these values were not determined.

SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Further Analysis of the Zero-length Crosslinking Reaction Between C4b and Eap34. *A*, Time-dependent, zero-length crosslinking control experiment using C4b alone. C4b was activated by treatment with EDC and NHS, prior to addition of buffer (0.1 M sodium phosphate (pH 7.5)) as described in *Materials & Methods*. Aliquots were withdrawn over the course of 180 min, and the contents were denatured, reduced, and separated by SDS-PAGE. No time-dependent changes were visible for either the α', β, or γ-chains of C4b. A representation of the C4b structure identical to that shown in Fig. 3C is shown here for ease of interpreting the gel image. *B*, The adduct band of intermediate molecular weight resulting from the 180 min time point in **Fig. 3A, *red box*** was further characterized. In-gel digestion of the excised band by trypsin generated a series of peptides that were mapped to Eap34 and C4b. Observed residues appear in red letters, while unobserved residues appear in black letters. 200 of 211 residues in the recombinant form of Eap34 were observed, for a total coverage of 94.8%, while 158 of 291 residues in the C4b γ-chain were observed, for a total coverage of 54.3%. Interestingly, the Eap34 peptide NLTSQIK, which contains the highly-protected K304 **(Figs. 1A and 4)**, was not observed. This suggests that this particular lysine was not accessible to the protease during the in-gel digestion procedure. Furthermore, a large region of peptide encompassing the C4b γ-chain was not observed, including several residues in the vicinity of the C terminus. These residues contribute a portion of the MIDAS-acceptor site, and are near the proposed binding site of Eap4 in the model of the C4b/Eap34 complex (**Fig. 7 and Supp. Fig. 4)**. One potential explanation for the absence of these peptides is that they represent a location of chemical crosslinks between sidechains of Eap34 and the C4b γ-chain.

Figure S2. Surface Plasmon Resonance Sensorgrams for MBP-C4b-C345c binding to EapFL/Eap34. Binding of an MBP-C4b-C345c fusion protein to either a full-length Eap (*A*) or Eap34 (*B*) surface was investigated by SPR. Injection of a logarithmic concentration series of fusion protein demonstrated clear evidence of binding to both surfaces, although saturation could not be achieved due to limitations of fusion

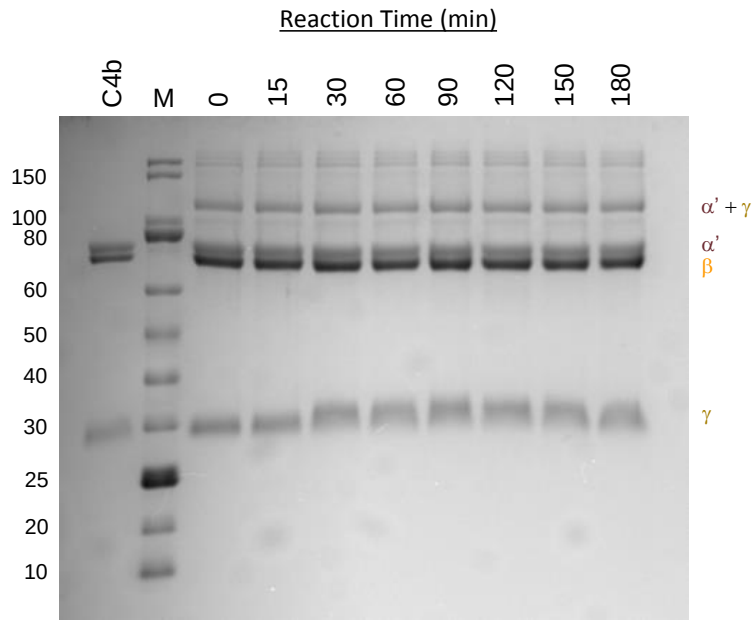
protein solubility. Each injection was performed in triplicate, and all reference-corrected sensorgrams are shown.

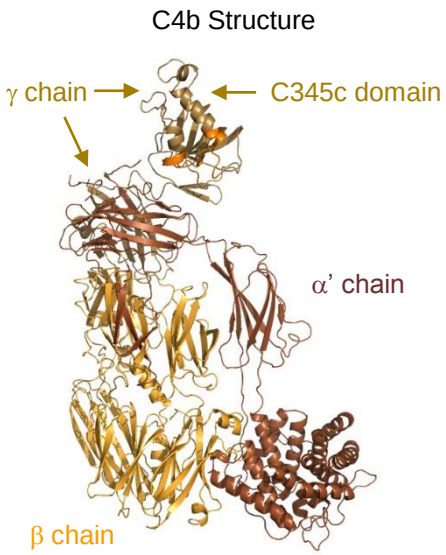
Figure S3. Supporting Data for Lysine Acetylation/Protection Studies. *A,* Chymotrypsin-derived peptide coverage maps from three independent experiments on Eap34, Eap34+NHS-Ac, and C4b/Eap34+NHS-Ac. Coverage maps for Eap34 ranged from 79.6 to 84.4%. Observed residues appear in red letters, while unobserved residues appear in black letters. The asterisk signifies that the first three residues in the recombinant Eap34 protein (i.e. GST) are an artifact of the subcloning procedure, and are not found in the full-length Eap sequence. *B,* Example changes in Eap34 chymotryptic peptides in samples of Eap34 alone, Eap34+NHS-Ac, or C4b/Eap34+NHS-Ac. The M/z of the peptide ions is shown along with each peptide identity. The three columns at the right depict the number of acetylations in each peptide that account for the observed increase in M/z. Of note, the third peptide (157-166) contains three lysine residues, but was only found to contain one acetylation in the C4b/Eap34 sample. Thus, the level of protection assigned to each lysine is 1/3 or 33%. A similar analysis was conducted across all observations to arrive at the data presented in **Fig. 4A**.

Figure S4. Supporting Images for the Model of the C4b/Eap34 Complex. The crystal structure of C4b (23) and a SAXS-derived model of Eap34 (15) were used to generate a structural model of the C4b/Eap34 complex via the ClusPro server. (32, 33) Additional biochemical constraints were derived from experimental data as described in *Materials & Methods*. Two rotated views of the C4b/Eap34 complex are shown with C4b drawn as a molecular surface (see **Fig. 7**) and Eap34 drawn in purple ribbon (see **Fig. 4B**). Protected lysine residues are highlighted in magenta (Eap3) and cyan (Eap4). The C4b surface is colored as follows: α'-chain in brown, β-chain in yellow-orange, and γ-chain in beige. The metal-ion dependent adhesion site (MIDAS) acceptor surface, which mediates binding of the vWF domain of C2 to the C345c domain of the C4b γ-chain, is colored orange for reference.

Woehl et al., Supplemental Figure 1.

A Zero-Length Crosslinking of C4b (Control)

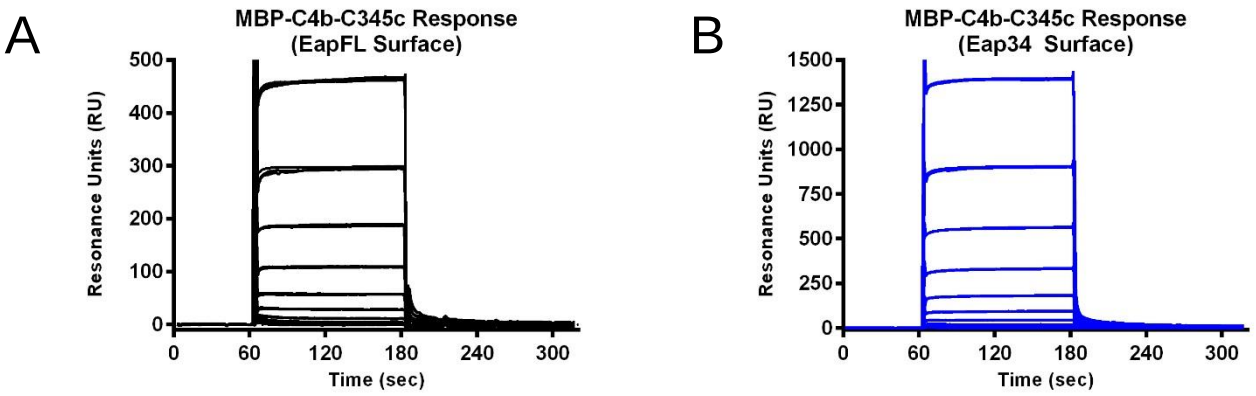




B Composition of Putative C4b + Eap34 Adduct Band (Trypsin Digest)

Sequence	Identity	Coverage (%)
GSTVPYSINL NGTSTNILSN LSFSNKPWTN YKNLTSQIKS VLKHDRGISE QDLKYAKKAY YTVYFKNGGK RILQLNSKNY TANLVHAKDV KRIEITVKTG TKAKADRYVP YTIAVNGTST PILSKLKISN KQLISYKYLN DKVKSVLKSE RGISDLDLKF AKQAKYTVYF KNGKKQVVNL KSDIFTPNLF SAKDIKKIDI DVKQYTKSKK K	Eap34	200/211 (94.8%)
EAPKVVEEQE SRVHYTVCIW RNGKVGLSGM AIADVTLLSG FHALRADLEK LTSLSDRYVS HFETEGPHVL LYFDSVPTSR ECVGFEAVQE VPVGLVQPAS ATLYDYYNPE RRCSVFYGAP SKSRLLATLC SAEVCQCAEG KCPRQRRALE RGLQDEDGYR MKFACYYPRV EYGFQVKVLR EDSRAAFRLF ETKITQVLHF TKDVKAAANQ MRNFLVRASC RLRLEPGKEY LIMGLDGATY DLEGHPQYLL DSNSWIEEMP SERLCRSTRQ RAACAQLNDF LQEYGTQGCQ V	C4b γ-chain	158/291 (54.3%)

Woehl et al., Supplemental Figure 2.



Woehl et al., Supplemental Figure 3.

A Chymotrypsin Coverage Map of Eap34 in Various Settings

Sequence (Eap34, residues 265-476*)	Sample	Coverage (%)
GSTVPYSINL NGTSTNILSN LSFSNKPWTN YKNLTSQIKS VLKHDRGISE QDLKYAKKAY YTVYFKNGGK RILQLNSKNY TANLVHAKDV KRIEITVKTG TKAKADRYVP YTIAVNGTST PILSKLKISN KQLISYKYLN DKVKSVLKSE RGISDLDLKF AKQAKYTVYF KNGKKQVVNL KSDIFTPNLF SAKDIKKIDI DVKQYTKSKK K	Eap34	175/211 (82.9%)
GSTVPYSINL NGTSTNILSN LSFSNKPWTN YKNLTSQIKS VLKHDRGISE QDLKYAKKAY YTVYFKNGGK RILQLNSKNY TANLVHAKDV KRIEITVKTG TKAKADRYVP YTIAVNGTST PILSKLKISN KQLISYKYLN DKVKSVLKSE RGISDLDLKF AKQAKYTVYF KNGKKQVVNL KSDIFTPNLF SAKDIKKIDI DVKQYTKSKK K	Eap34 + NHS-Ac	178/211 (84.4%)
GSTVPYSINL NGTSTNILSN LSFSNKPWTN YKNLTSQIKS VLKHDRGISE QDLKYAKKAY YTVYFKNGGK RILQLNSKNY TANLVHAKDV KRIEITVKTG TKAKADRYVP YTIAVNGTST PILSKLKISN KQLISYKYLN DKVKSVLKSE RGISDLDLKF AKQAKYTVYF KNGKKQVVNL KSDIFTPNLF SAKDIKKIDI DVKQYTKSKK K	Eap34/C4b + NHS-Ac	168/211 (79.6%)

B Example Changes in Peptides Due to Presence of NHS-Ac/Ligand

M/z	Peptide	Eap34	Eap34 + NHS-Ac	Eap34/C4b + NHS-Ac
1230.7416/1314.7627	^{32}KNLTSQIKSVL^{42}	0	2	-
2197.1975/2365.2398	^{148}KSERGISDLDLKFAKQAKY^{166}	0	4	-
1211.6783/1253.6888	^{157}DLKFAKQAKY^{166}	0	-	1
786.4396/828.4502/ 786.4396	^{134}ISYKYL^{139}	0	1	0

Woehl et al., Supplemental Figure 4.