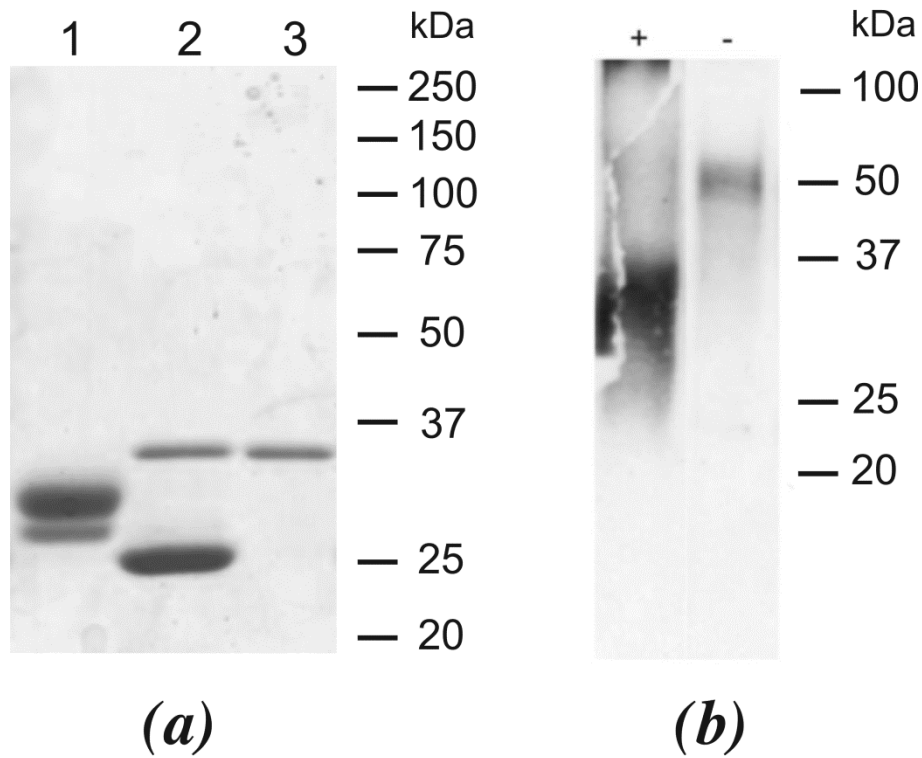


Supplementary Material



Supplementary Figure S1. Characterization and *N*-glycosylation status of purified recombinant *Oo*-ASP-1.

(a) 6 μ g of purified intact (lane 1) and PNGaseF treated (lane 2) *Oo*-ASP-1 were analyzed on a 12% SDS-PAGE gel, followed by Coomassie Brilliant Blue staining. For comparison, lane 3 contains only PNGaseF. (b) Purified recombinant *Oo*-ASP-1 migrates as a 30 kDa monomer on a 12% SDS-PAGE gel under reducing conditions (+) and as a 45-50 kDa dimer under non-reducing conditions (-). The amount of protein loaded on the gel was 6 μ g.

CAP3

61

Oo-ASP-1	1	-----GFCC-----PADLNQTD EAR KIFLDFHNVRRDLAGASPLLNLTGAVQMRNV-----	47	Group 4
Tc-ASP	1	-----GFCC-----PADLDQT EAR KILLNFHNEVRRDVSASAPLLNLTGAVLMRNV-----	47	

Na-ASP-1 (N)	1	-----MFSPIVIVSIFPTIAFCASPARDGFGC-----SNSGIT DKDR QAFLD FHN NARRRVAKGVEDSNSGK-----	62	Group 3
Ac-ASP-3	1	-----MKSYLIVISAALIGIAYADADYSKC-----PQNEIMNNDMREKV-TDMHNAYRSKFAR-----	51	
Ac-ASP-4 (N)	1	MINIHFI AL ALATSL L LALSEGKPVVFPQC-----KPNGLYHKNTIDNNVLPINT RR EALAKGTQQNGFPPNPQTF L -----	75	
Ac-ASP-4 (C)	277	STSSSTSASGATTTKAPSPQAPFTGTSTMCNTRHAYANRMT N NLRNEYVRLHNFRRGLLAKGEIPQKGNLYL-----	348	
Ac-ASP-5 (N)	1	-----MPNLLLLLFLSLPGAILSTTC-----PGNDLT DAERT LLTRVHNSIRREIAQGVANNYHGGKL-----	58	
Ac-ASP-5 (C)	219	-----HPPNPPNPFPAMS-----PNAEMT DAARK KVLGMHNWRRSQV AL GNVQNGKNAYNC-----	269	
Ac-ASP-6 (N)	1	-----MKLFLVLAALIGIAHATDFQC-----WNFKST DTL REHYLKSINLRK LA DGSAENKSGKC-----	58	
Ac-ASP-6 (C)	235	---PTTAPAPPTTAAPTTSTIGSIDNTIC-----PQNQVIT DS VRLTFLNTHNGLRSQ LA GGQIFMNGGARAR-----	301	
NIF	1	-----MEAYLVVLA LAG IAHSNEHLRCPQNGTEMPGFN DS IRLQFLAMHNGYRSK LA LGHISITEESESDDDDFGLPDPFA	79	

Na-ASP-2	1	-----GC-----PDNGMSEARQKFLMHNLSR S VALGQAKDGAGGNA-----	39	Group 1
Na-ASP-1 (C)	224	-----NQQC-----PSNTGMTSDVRDTFLSVHNEFRSSVARGLEPDALGGNA-----	265	
Ac-ASP-2	1	-----MLVLP L LALAVSVHGM S MRC-----GNGMTDEARQKFLDVHNSYRSMVAKGQAKDAISGNA-----	59	
Ce-VAP-1	221	-----FTTWC-----PSVTDQSDQARQNF L THNKR TS LAKGLEADGIAAGAF-----	265	

Ac-ASP-7	1	-----LDIVIDNQGS-----GC-----MVDPRFPEAIDTFHNLQR IA KAEAGYGPAREM-----	47	Group 2
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CAP4

1 2 *

Oo-ASP-1		LGPAKNMYRMDWD CN LEKAKAM W PC T PLFDITSIPQ N LAQ M LLFQNSQENEVL T Q P MSWV T ASLR N LQPDTEANIYNW-----	129
Tc-ASP		LGPAKNMYRMDWD CN LEKALEM S PC T VPLFDITSIPQ N LAQ M LLYR K ME E TEVLEKAP S WV I ASLR N LKNDTEADLYNW-----	129

Na-ASP-1 (N)		LNFAKNMYKLSWDCAMEQQLQDAIQSCPSAFAGIQ-----GVAQNVMSVSSSGGFPD P SVKIEQ T LSGWSGAKKNGVGPDKYNGGG-----	138
Ac-ASP-3		DHQASKMRKLVYDCAIEKGIYESDTKCEMK-----PSMEEENVEVDGNSD L TVISEA-----GNSWSEILD L KD V NSVDNT-----	121
Ac-ASP-4 (N)		PPATDMTKLSWCDLEQKALKTINGC V NFANPTKPNNGELAD V LYGNDYDNTVEGV I QGNLEAWLVKADFNVPV T TKGTVISYP-----	156
Ac-ASP-4 (C)		FKAADMWIKSYDCLEQGAIEHASQCLTGGSGQSRPVGGEN F KVIPAARF P FEDA A AKKT-VTE W KPIRVVDYFGNNVFLPIY-----	426
Ac-ASP-5 (N)		PAGKNIYRMRYDCELEQAADASQ T CSASLEEPQKYGQNIQAYV T PSIARPKNDLLEDA--VKQWYLPV I YGGORDAANKFTD P R L Y-----	138
Ac-ASP-5 (C)		PTATDMYKLEDCLENSAL-AYAKC S LVGSAE C TRPGE V ENHVKALV T DEAAVQ T A--VQAWWSQISQ N GLNAQMKFTAP L KD-----	346
Ac-ASP-6 (N)		PQKNIYKLSWCELEKQAQAV-DQCKPNVPEFA-----GYSQILK K VK T CD P TKVLKQ-----IEAW T SKV K DAGVDNPN N KQ-----	130
Ac-ASP-6 (C)		RFASKMRMYNVCDAESSAR-NSAAG C LSSPGSPS--GYTENLHV I NNNVDHNSAATQ A --PNAWSE I NTGYMRQ A ETERM Y SLSVGI	380
NIF		FRASKMRYLE-YDCEAKSAYMSARNCS S SSPP--EGYDEN K YIFENS N I S EALKA--MISWAKE A FN L NK T KEG V LYRS-----	152

Na-ASP-2		PKAAKMTMA-YDCEVERTAMNNAK C VFKHS Q PNQRKGLGENIF M SSDSGMDKAKAEQA--SKAWFGE L A E KGVGNL K L T GG L FS-----	117
Na-ASP-1 (C)		PKAAKMLMV-YDCEVEASAIRHNK C VYQHS G EDRPLGENIY K TSV L K F DKNAKQA--SOLW N ELKE F GVG P SNV L TALW--N	343
Ac-ASP-2		PKAAKMKMI-YDCNVESTAMQNAK C VFAHSHRK--CVGEN I W M STARQMDKQAQAQQA--SDCWF S ELAK V GVG E NK L T T QLW-----	140
Ce-VAP-1		PHAKMFKLVKYSCTVEANARTWAK C CLYQ H STSAQRPLGENL M ISIN N M P KIQ T A B DS--SKAWSE L K D FGV G SDN I L T QAVF-----	350

Ac-ASP-7		YGL-----YDCGLEEEARKEIKL-----PGYADL H HRGV T RFSGD-----YEGSAISALKEI L ET T FA-----	105
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CAP1 **CAP2** ### (G-X-P/V motif)

Oo-ASP-1		* 3 2 3 4 5 4	
		---QIRPLS--NIANQN L KVGC A HKVC-----KFFTGT N NVVSCAYGGEVLQDNEVVWDK G PT---CH---CNAYPNS F -----CCN	196
Tc-ASP		---KIRTTIS--NLLNWR N KVGC A HKVC-----GFPTGT N NVVIS C AYGDKLENNEVVWG K PT---CE---CNAYPDSY-----CCN	196

Na-ASP-1 (N)		---LFAFS--NMVSEY T KLGC A V F VC-----NDFTFG V QVQY F PGGNYM G HVIY T MGQP---CSQ---CSPGAT-----CSV	211
Ac-ASP-3		---SEIA--NMAESHAK L GC A V F VC-----SKK T HV C RYG P EGKGEK K IY E KET---CSQ---CSDY G QGV T -----CDN	191
Ac-ASP-4 (N)		TYNGTDL L AYS N LVRPTNTEIG C VLERC-----PATANV P L V TF P CI L NGKNITNGEALYK G T V NTGG K EV T --CSAGYA-----C N N	243
Ac-ASP-4 (C)		---DQDP I SS F TRMAWATTN K VGCS I V F C-----T D NVYV G VC R YSPMGN I VNSNIYQ I GNP---CSV---RPTQATG-----C D P	501
Ac-ASP-5 (N)		---TFA--NLAYDKNTALG C H Y AF C -----QGPDR I VIS C MYNNV P DN V IYEPG T A---CVK D AD C T T Y P Q S T-----C K D	210
Ac-ASP-5 (C)		---KDPAPT A FT--QMAW A SKV K LG C A V S N C-----QAD T F T V C R Y K A AGNI V G E F I Y T KGNV---CDA---CKAT-----C I T	416
Ac-ASP-6 (N)		---LEDFA--KLANGK A K I GC A Q K NC-----NEQLY V AC V INEPAP A V G MP I YEV G AG---CNS K DD C T T Y L Q S K-----C S N	203
Ac-ASP-6 (C)		---PNFA--KMAWET N AH L GC A V F VC-----GLNT N V V CV P YS F KSDG Q IY K MG P F---CRR---CPDY P GT F -----C N Q	447
NIF		---NHDISNFA--NLAWDARE K F G CA V V N CPLGEID E T N H D GET Y A T TH V V C H Y PK I N T EG Q IY R VG T P---CDD---CSEY T K R AD N T S AD P V C IP	250

Na-ASP-2		---RGVGHYT--QMVVQ E T V KLGC V Y E AC-----SNMCY V VQ V QY G PAGN M GK D IY E K G EP---CSK---CEN-----C D K	184
Na-ASP-1 (C)		RPQMIG H YT--QMAW D TT T KLGC A V F VC-----G T K L A V S C I N GV G Y I T N Q P MW E T G QA---C H T G AD C S T Y K NS G -----C E D	416
Ac-ASP-2		NRGVMI G HYT--QMVVQ E S Y KLGC V Y E VC-----SSMTY V G V Q V YS P QGN M NS L IY E K G PN---C T K D S C GS N AS-----C S A	209
Ce-VAP-1		---DRGVGHYT--QMAW E GT E IG C F V EN C -----PT F T S V S V Q Y G PAGN M N Q L I Y T K G SP---C T AD A D C FG T Q T -----C S V	417

Ac-ASP-7		---DNKSMR--QVYV P K A TR F GC S GR L R R NK T G-----MRR M D V CV Y D K K P KD--GES F E G G K P---C N E N K D C T Y Y K G S T -----C E W	172
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5 6

Oo-ASP-1		---NLCD T IAA A TLRN Q PK S T-----	215
Tc-ASP		---NLCD T KAA A ALRE E PCK S N-----	215

Na-ASP-1 (N)		---GLCTK G P D V P ET-----	223
Ac-ASP-3		DEWEG L LCS-----	200
Ac-ASP-4 (N)		---ATLLCERS A TTSS S TAS S TS S AS S SS S MAI	276
Ac-ASP-4 (C)		VEGLWY-----	507
Ac-ASP-5 (N)		---SLC I I P TP-----	218
Ac-ASP-5 (C)		---AEG L C P TP-----	424
Ac-ASP-6 (N)		---KVCV A GH P GDAT T TT S TP A TT A PT T PI P AG	234
Ac-ASP-6 (C)		---GLCS F -----	452
NIF		---DDG V CF I GS K AD Y S K E F Y R F R E L -----	274

Na-ASP-2		---EKGLCSA-----	191
Na-ASP-1 (C)		---TEGLCSA P -----	424
Ac-ASP-2		---GEALCV V R G -----	218
Ce-VAP-1		---A E ALCV I P-----	425

Ac-ASP-7		---NLCY T TF-----	179
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Supplementary Figure S2. Secondary structure-based amino acid sequence alignment of several ASPs and other CAP proteins (based on Osman et al., 2011). PSIPRED was used to predict secondary structures, which are shown in green (α -helix) and blue (β -strands). CAP sequence motifs 1 to 4 are shown above the sequences, whereas cysteine residues are numbered according to the disulfide bridge to which they belong. Conserved histidine residues (only for Group 1 ASPs) are indicated by an asterisk. For *Oo*-ASP-1, the cysteine residue involved in dimerization (Cys195) is shown in red, whereas the cysteine residues (Cys3 and Cys212) involved in the sixth dithioether bond are in italic. Neutrophil inhibitory factor (NIF) and the *Caenorhabditis elegans* VAP-1 were included for comparison.