

A

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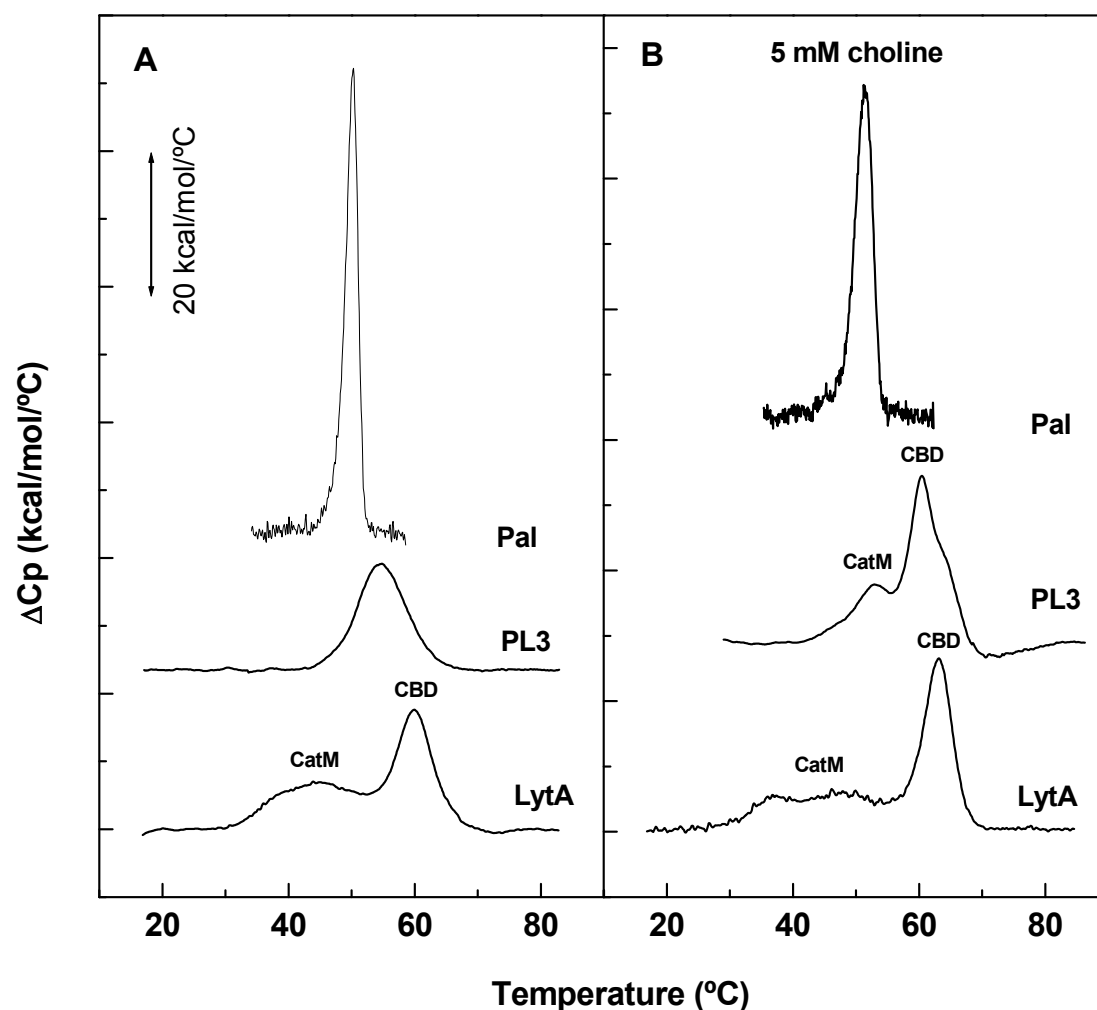
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      60      70      80      90     100
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     110     120     130     140     150
GMFIDSDNII HCNAYDGIS VNDHDERWYY AGQPYYVYR LTNANAQPAE
     160     170     180     190     200
KKL GWQKDAT GFWYARANGT YPKDEFEYIE ENKSWFYFDD QGYMLAD RWR
     210     220     230     240     250
KHTDGNWYWF DNSGEMATGW KKIADKWYF NEEGTMKTGW VKYKDTWYYL
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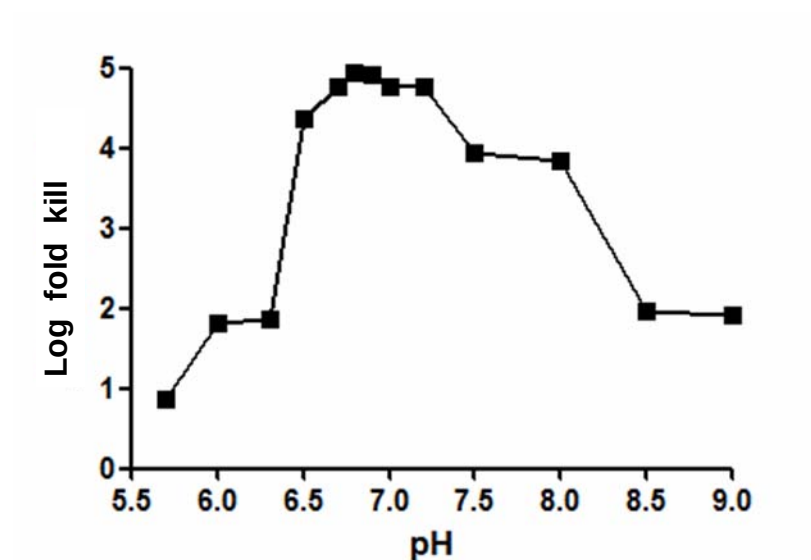
B

Net charge				Protein	Specific Activity (U/mg) × 10 ⁻⁵	Choline IC ₅₀ (mM)	Origin
Z _{CatD}	Z _{linker}	Z _{CBD}	Z _{total}				
-4.7	1.0	-7.8	-11.5	PL3	4.0 ± 0.9 ^a	9	Synthetic
-4.7	1.0	-6.8	-10.5	Pal	1.2 ± 0.1 ^b	2	Lytic phage
-8.0	-1.9	-4.7	-14.6	LytA	2.5 ± 0.2 ^b	24	<i>S. pneumoniae</i> R6

Figure S1. Amino acid sequence and characteristics the PL3 chimera. (A) The one letter amino acid code is highlighted according to the color code used in **Figure 1** to identify the origin of the catalytic domain (CatD; Pal, green), linker (Pal, violet), choline binding repeats of the CBD (Pal, grey; LytA, blue) and the C-terminal tail of the latter. The last six amino acids of the second repeat (GYMLAD) are common to the two parental enzymes. Cysteine residues are underlined. (B) Net charge, specific activity, half choline inhibitory concentration (IC₅₀) and origin of PL3 and the parental lysins. Z_{CatD}, Z_{link}, Z_{CBD}, and Z_{total} correspond, respectively, to the net charge of the CatD, the linker, the CBD and the total net charge. ^aMeasured at 37°C in PB, pH 6.8, 10 mM DTT. ^bData from Sheehan et al. (1997).



SUPPLEMENTARY FIGURE S2 I Thermal stability of PL3, Pal and LytA. Comparison of PL3 and parental murein hydrolases thermal stability in the absence (A) and in the presence (B) of 5 mM choline. Thermograms registered in PB, pH 8.5 (PL3) or pH 8.0 (Pal^a and LytA^b); curves were shifted along the y-axis for clarity (CatD and CBD indicated the position of transitions assigned to the catalytic domain and the choline-binding domain, respectively, when appear resolved in the thermograms). ^aData from Varea et al. (2004). ^bData from Varea et al. (2000).



Supplementary Figure S3 I pH dependence of PL3 bactericidal activity. Exponentially growing R6 cells were washed and suspended in PB containing 100 mM NaCl and 10 mM DTT, at different pHs, in the presence of 0.1 $\mu\text{g/ml}$ of PL3. Incubation continued for 60 min at 37°C. Then, viable cells were counted and killing activity was calculated as the difference between bacterial titers (CFU), in log units, of PL3-treated and untreated controls.

SUPPLEMENTARY TABLE S1 I Parameters of major forms of PL3, LytA and Pal

	PL3			LytA	Pal	
	monomer	dimer	tetramer ^a	dimer	monomer	dimer
Molecular mass (kDa)	34,151	68,302	136,604	73,088	34,453	68,906
Sedimentation velocity						
$s_{20,w}$ (S)	3.0	4.1	6.8	4.1	3.3	4.2
ff_0	1.25	1.51	1.38	1.56	1.27	1.52
R_s (Å)	27.2	41.5	47.9	43.9	27.8	41.0
Choline titration^b						
K_1^{app}	60 ± 9 (μM)			1.1 ± 0.5 (mM)	8 ± 3 (mM)	
p_1^{app}	2.1 ± 0.7			1.0 ± 0.2	0.8 ± 0.2	
K_2^{app}	4.5 ± 0.6 (mM)			6.8 ± 0.4 (mM)	9.7 ± 0.5 (mM)	
p_2^{app}	0.9 ± 0.1			5 ± 2	7 ± 2	

^aParameters calculated for the tetramer without choline. ^b K_1^{app} and K_2^{app} are the apparent half dissociation constants for the higher and lower affinity sites, respectively, estimated by fitting two sigmoid functions to the experimental data. p_1^{app} and p_2^{app} describe the sigmoid slope.