The antigenic identity of human class I MHC phosphopeptides is critically dependent upon phosphorylation status

Supplementary Materials

Supplementary Table 1: Crystallisation, data processing and refinement statistics

	PKD2		RQA_V		RQI	
PDB ID CODE	4NNX	4NNY	4NO2	4NO0	4NO3	4NO5
Peptide sequence	RQApSLSISV	RQASLSISV	RQApSIELPSMAV	RQASIELPSMAV	RQIpSQDVKL	RQISQDVKL
Peptide source	PKD2	PKD2	LSP-1	LSP-1	AMPD2	AMPD2
Crystallisation						
Precipitant (%) Buffer (0.1 M) Salt (M) Additive (0.003 M)	PEG 3350 (11) HEPES pH 7.5 NaCl (0.15) $MgCl_2$, $CdCl_2$	PEG 3350 (15) HEPES pH 7.5 NaCl (0.2) $MgCl_2$, $CdCl_2$	PEG 8K (20) HEPES pH 7.5 - -	PEG 3350 (18) HEPES pH 7.5 NH4-Acet (0.2) -	PEG 3350 (12) Tacsimate pH 8 - -	PEG 3350 (19) Bis Tris pH 6.5 Na tartrate (0.1) -
Protein (mg/ml)	10	10	17	10.5	11	11
Data Processing						
Resolution range (Å)	20–2.1	20–1.9	20–2	20–2.7	20–1.7	20–2.1
Unit cell Parameters a , b, c (Å) α, β, γ (°)	 61.1, 79.8, 111.3 90, 90, 90	 60.4, 79.8, 110.7 90, 90, 90	 119.2, 54.7, 75.2 90, 104.8, 90	 117.3, 117.3, 96 90, 90, 120	 118, 54.2, 75.1 90, 104.9, 90	 56.4, 79.3, 56.8 90, 115.5, 90
Space Group	$P2_12_12_1$	$P2_12_12_1$	C2	P3121	C2	$P2_1$
Total reflections	228414	346902	317693	773823	334999	299127
Unique reflections	31648	42471	31594	21363	46573	26307
Multiplicity	7.2	8.2	10	36	7.2	11.4
Completeness (%)[a]	99 (96.4)	99 (94.6)	98.9 (96.1)	98.9 (99.5)	91.9 (75.4)	99.3 (98.8)
R_{merge} (%)[b]	10.8 (38.5)	9.2 (52.9)	5.4 (46.4)	12.6 (81.6)	3.4 (32.8)	8.8 (24.0)
I/σ(I)	15.3 (4.8)	16.6 (4.9)	31.6 (4.5)	33.2 (6.2)	34 (4.7)	25.2 (11.4)
Refinement						
Resolution (Å)	20–2.1	20–1.9	20–2	20–2.7	20–1.7	20–2.1
Reflections used	31643	42469	31330	21363	44256	25030
R_{cryst} (%)[c]	19.8	20.8	20.8	26.9	20.8	18.8
R_{free} (%)[d]	23.6	22.8	24.9	30.9	23.2	23.7
Protein residues	382	382	386	580	383	382
Water molecules	211	301	258	51	391	266
RMS deviations Bond lengths (Å) Bond angles (°)	 0.016 1.46	 0.009 1.07	 0.010 1.17	 0.018 1.63	 0.0082 1.11	 0.0096 1.14

Figures in parentheses in the data processing section apply to data in the highest resolution shell. [a]Completeness = (number of independent reflections/total theoretical number). [b]R_{merge} (I) = (Σ|I(i) - <I(h)>|/ΣI(i)), where I(i) is the ith observation of the intensity of the hkl reflection and <I> is the mean intensity from multiple measurements of the h,k,l reflection. [c]R_{cryst} (F) = $\Sigma_h||F_{obs}(h)| - |F_{calc}(h)||/\Sigma h|F_{obs}(h)|$ and $|F_{calc}(h)|$ are the observed and calculated structure factor amplitudes for the h, k, l reflection. [d]R_{free} is calculated over reflections in a test set not included in atomic refinement (5% of data). Peptides are derived from PKD2 (protein kinase D2), LSP-1 (lymphocyte specific protein 1) and AMPD2 (adenosine monophosphate deaminase 2).

Supplementary Table 2: Comparison of phosphate-independent stabilising contacts mediated by RQA_Vp and RQA_Vnp peptides and HLA-A*0201

	RQA_Vp	RQA_Vnp
Peptide	**HLA-A2**	
R1	Tyr159 (3)	Tyr159 (3)
Q2	Tyr159 (5)	Tyr159 (6) **Q2 (O) - Y159 (OH) 2.8Å**
A3	Tyr159 (14)	Tyr159 (8)
S4/p-Ser	(See Table S3)	-
I5	Gln155 (11) Leu156 (5) Intra M10 (1)	His70 (21) Thr73 (3) **I5 (O) - His70 (N) 2.9Å** Arg97 (7) Tyr99 (1) Intra M10 (3)
E6	-	Ala69 (5) Thr73 (4)
Total contacts	39	61

Values in parentheses correspond to the number of hydrophobic and van der Waals contacts mediated between the peptide and HLA-A2. Hydrogen bonds with donor to receptor distances of 2.3-3.4Å are shown in bold. Intra corresponds to an intra-peptide stabilising interaction between I5 and M10.