Evaluation of Endosomal Protonation on Peptide Binding to Class II Major Histocompatibility Complex Molecules

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Introduction

Recognition by CD4+ T cells of pathogen-derived antigenic peptides bound to class II MHC molecules (MHCII) and expressed on the surface of antigen presenting cells (APCs) is a crucial step in the initiation of an adaptive immune response. The ability to predict whether a peptide sequence binds to an MHCII can aid the development of vaccines, as well as provide the biochemical and biophysical underpinning to the measurement of immune response.

Current informatics systems employed to predict MHCII/peptide presentation were developed on the basis of a rigid docking model and in conditions emulating the extracellular environment at pH 7.4. These methods suffer from low accuracy, most likely because they ignore the flexibility of the peptide/MHCII system, as well as the varying levels of acid pH the complex experiences within the endosome, where the peptide binding reaction usually occurs. Therefore, using the human allele HLA-DR1 (DR1), and a library of peptides derived via cycle mutation (Table I) from the sequence of HA peptide (H3 strain) residues 306-319, we have evaluated the impact of solvent protonation on peptide/DR1 (pDR1) complex flexibility, measured as cooperativity, with the long-term goal of developing an accurate informatics system to predict MHCII/peptide binding affinity.

			P1	P2		P4		P6	P7		P9		
G	P	K	Y	V	K	Q	N	T	L	K	L	A	T
				V	D		E			G			

Table I - The sequence of the HA306-319 peptide from H3N2 influenza virus is indicated in the second row. The peptide binds through encapsulation of hydrophobic side-chains in polymorphic pockets located at the extremities of the HLA-DR1 binding groove (P1 and P9). Shallower pockets are lining the groove (P2, P4, P6 and P7) and interactions at these positions also contribute to the binding. Finally, there is an extensive H-bond network between side chains of non-polymorphic residues in the DR1 alpha helixes and the peptide backbone (not shown). The substitutions applied via cycle mutation are indicated in the third row.

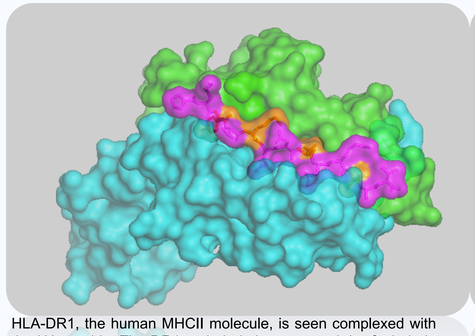
Materials and Methods

Peptides were derived from the sequence GPKYVKQNTLKLAT, representing residues 306-319 of the hemagglutinin protein from influenza A virus (H3 subtype). The N-terminal Gly facilitated labeling. N-terminal labeling with or LC-LC biotin (Pierce) was performed (Anaspec, CA).

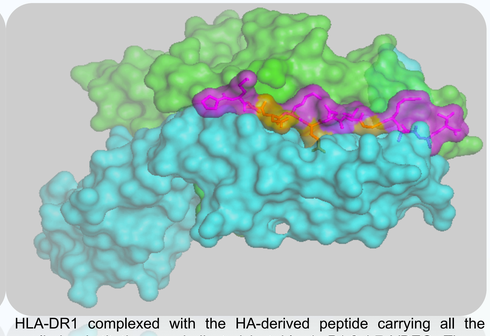
Recombinant soluble empty (peptide free) DR1 was produced and purified by ion-exchange chromatography from a stably transfected *CHO* cell line. DR1 proteins were quantified by measuring the UV absorbance @ 280 nm using an E_{280} of 56340 M^{-1} cm^{-1} before use(Amicon, Pierce).

DR1 (20 nm) was incubated with 20 nm biotinylated HA peptide in PBST (pH7.4), MES (pH6.4), and Sodium Citrate (pH5.4) in the presence of varying amounts of inhibitor peptides at 37°C. Bound biotinylated peptide was detected using a solid-phase immunoassay and Eu^{2+} labeled streptavidin. Plates were read using a Perkin Elmer Victor X5. IC_{50} values were obtained from the curve fit of the binding data and converted to K_D values by using the Cheng-Prusoff equation $K_D = (IC_{50})/(1 + [bHA]/K_{D,bHA})$). Each point represents the mean and SD of three independent experiments (unless otherwise specified) performed in quadruplicate.

For calculating cooperativity, the effect of multiple substitutions is measured directly (observed value). The expected value for a combination of substitutions is calculated as the product of the peptide Kd fold changes resulting from single substitutions as compared to wtHA [e.g. $\Delta K_{d,exp}\,x,y = (\Delta K_{d,}\,x)$ x $(\Delta K_{d,}\,y)$]. The cooperativity is the ratio of the expected to observed (C = obs/exp) values for ΔK_d.



HLA-DR1, the human MHCII molecule, is seen complexed with the HA peptide. The DR1 α-chain is in green and the β-chain in blue. The magenta peptide residues interact with the shallow shelves of the DR molecule, orange peptide residues conversely interact with the deeper pockets P1, P4, P6, and P9 of the DR molecule.



HLA-DR1 complexed with the HA-derived peptide carrying all the applied substitutions as indicated in table 1: P1,2,4,7 VDEG. These substitutions are not conservative and result in decrease of peptide binding. Graphically modeled residue substitutions of P1,2,4,7 VDEG suggest a reduced surface interaction between the peptide and the DR1 binding groove, likely resulting in increase of solvation and complex destabilization.

Results

Peptide affinity was measured using an equilibrium-based competition-binding assay in which each peptide was tested for its ability to compete against the HA peptide for binding to DR1. This allowed us to examine cooperativity in highly unstable multisubstituted complexes. The binding curves at pH 5.4, 6.4 and 7.4 for all the complexes are shown below (Figure A - C). Each individual substitution resulted in small to negligible effects on the dissociation constant whereas multiple substitutions had significantly larger effects. This large increase in dissociation constant suggested a cooperative effect, and it appeared to be a function of pH (Figure D). To test this possibility, the relative effect of each singly substituted complex was calculated with respect to the peptide affinity for the unsubstituted DR1/HA complex. If the contribution of each substitution to complex stability was independent, then the effect of multiple substitutions should equal the product of their individual effects on stability. The ratio of expected to observed stability gives the cooperativity. The data clearly indicate the presence of cooperative effect between the peptide and the MHCII in generating the final complex, and protonation impacts the magnitude of the phenomenon. Plotting the cooperativity values and K_d for each multisubstituted complex on a ln scale revealed an increasing cooperativity with decreasing K_d, as the data can be fit to a linear regression with a positive slope, and the slope values appear to be correlated to pH binding conditions (Figure E).

Figure A

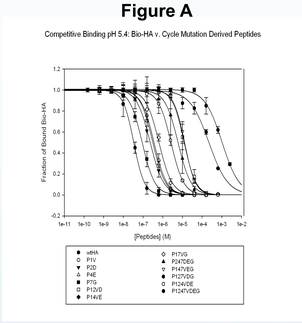


Figure B

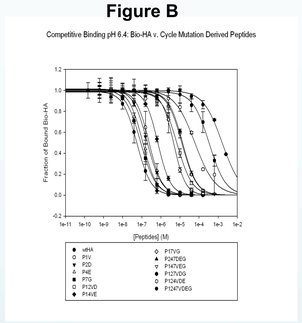
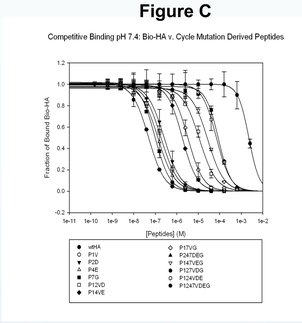


Figure C



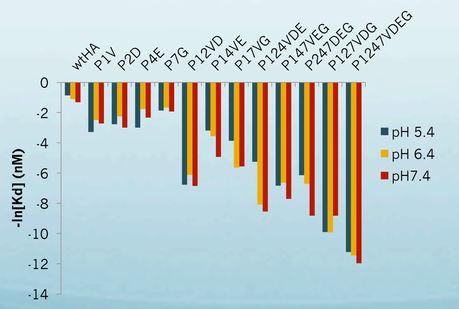


Figure D

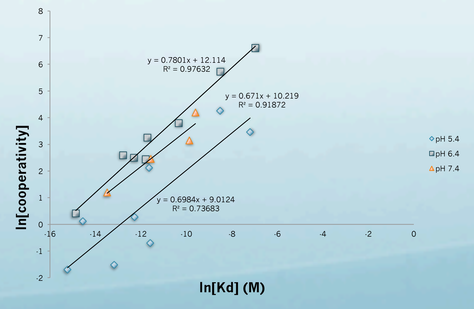
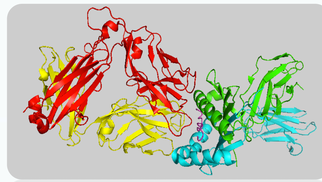


Figure E

Discussion

Cooperativity in a multipoint ligand-receptor binding event is evidenced by a disproportion between the observed affinity value and the expected value based on multiplication of affinity values of single residue exchange. A general strategy to investigate the occurrence of such a phenomenon is the mutant cycle approach. This consists in introducing multiple substitutions in the sequence of peptides and assessing their binding abilities. If the effect on the binding affinity of the double (or triple) mutation is not equal to the product of effects of the single mutations, then the two (or three) residues are coupled (cooperative). We are applying this strategy to determine the extent of cooperativity the pDR1system undergoes during formation. We began our analysis of cooperativity in peptide binding to DR1 by measuring the affinity of the wtHA peptide and the peptides with single substitutions at pH 5.4, pH 6.4, and pH 7.4, then transitioning to peptides with multiple substitutions. Results of these initial experiments reveal that multiple substitutions in the peptide sequence greatly affect the binding affinity of the peptide to DR1, most notably in acidic conditions, where binding affinity can be altered by an order of magnitude (Table II).



pDR1 is shown complexed with a T-Cell Receptor (TCR). The α and β chains of the TCR are respectively in red and yellow. Blue and Green represent the α and β chain of DR1. Represented by the color magenta, is the HA peptide.

Peptide	pH 5.4 K_D (nM)	pH 6.4 K_D (nM)	pH 7.4 K_D (nM)
wtHA	2.39	3.02	3.71
P1V	26.8	12.1	10.4
P7G	6.42	5.29	6.88
P17VG	47.8	282	258

Table II – Reported are affinity values for three peptides as exemplification of the effect of pH on binding strength.

Presently, our results indicate that a trend exists between solvent protonation and peptide binding to DR1, though this effect is not consistent, rather dependent on the position and nature of the substitution. Because some peptides have been assayed only in a single experiment we are not yet able to asses statistical significance of the observed differences. However, as cooperativity is a surrogate measurement of folding, and a correlation between solvent protonation and protein folding has been extensively show in other systems, we expect to observe a confirmation of this initial trend. Any result will provide important insights to understand the effect of pH on antigen presentation by MHCII molecules and it will impact our ability to predict peptide binding and epitope selection.

Acknowledgements

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