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

Kyle L. Crane, Marie Gotti, Megan Templeton, J. Margaret Castellini* & Andrea Ferrante, Department of Biology and Wildlife
Institute of Arctic Biology
School of Fisheries and Ocean Sciences

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 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 binding presentation suffer from low accuracy, most likely because they ignore the complex flexibility, measured as cooperativity, of the peptide/MHCII system, as well as the varying levels of acid pH the complexes experience 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.

Table I - The sequence of the HA306-319 peptide from H3N2 influenza virus is indicated in the second row. Results through encapsulation of hydrophobic side-chains in polymeric pockets located at the extremities of the HLA-DR1 binding groove (P3 and P9). Shallow pocket lines 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-polymeric residues in the DR1 alpha helices 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 GPKTVKQNTELKLAVD 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, USA).

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 at 280 nm using an E280 of 56340 M-1 cm-1 before use (Amicon, Pierce).

DR1 (20 μM) was incubated with 20 μM biotinylated HA peptide in PBST (pH 7.4), MES (pH 5.4), and Sodium Citrate (pH 5.4) in the presence of varying amounts of inhibitor peptides at 37°C. Bound biotinylated peptide was detected using a solid-phase immunosassay and biotinylated streptavidin. Plates were read using a Perkin Elmer Enzyme Immunoassay and Eu-labeled peptides at 37°C. Bound peptides were measured by using the Cheng-Prusoff equation Kd = (IC50) / (1 + [HA]) (Kd/IC50). 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 wild type (e.g. ΔKdobs = (ΔKd1) x (ΔKd2) x (ΔKd3)). The cooperativity is the ratio of the expected to observed (ΔKdobs/ΔKdexp) values for ΔKd.