MHC Class II-Peptide Complexes: H/D Exchange study by LC-MS/MS Analysis.

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One of the most interesting problems in immunology is activation of T cells by antigens. The activation involves extra and intracellular protein degradation by proteases. Peptides representing partial sequences find their way to the cell surface in association with the major histocompatability complexes (MHC) Class I or II. Peptides in these complexes are recognized by T-cells as self or no-self.

Major Histocompatibilty Complex (MHC) class II proteins are heterodimers (m.w. aprox. 60000) expressed in specialized antigen presenting cells such as dendritic cells, macrophages and B lymphocytes (APC). These proteins bind peptides generated by catabolism of antigens in endosomal compartments. These protein/peptide complexes migrate to the surface of the APC for CD4+ T-lymphocyte recognition, eventually triggering a cellular immune response.

The crystal structures of some MHC class II have been determined[1]. They show that the binding site is composed of an eight stranded anti parallel β-sheets as the floor and two anti parallel long helices as the sides. The peptide is stretched in the binding site forming a network of hydrogen bonds with the MHC protein. Moreover, high-energy interactions between peptide side chains and deep pockets in the binding site account for the binding specificity. Strong interaction of the peptide with the binding site is likely to be physiologically important, it allows the complex to persist and increase its immunogenicity.

As part of an ongoing study[2] on the mechanism of antigen processing before presentation by the MHC class II, we are probing the solution structure of MHC II protein-peptide complex. X-ray crystallography can give very detailed information about protein three-dimensional structure. However, the requirement to crystallize the protein, limits its applicability. Moreover the protein crystal structure doesn't always reflect the solution dynamics of the protein. Multi dimensional NMR can be used also to determine protein structures in solution but it is usually applicable to small proteins under 20 kDa. Another important method is protein backbone amide hydrogen exchange. The exchange rate of a hydrogen is closely related to its local environment. Another factor which determines amid hydrogen exchange rate is pH. The minimum rate for most amino acids amid hydrogen is about pH 2.75 while the rate for pH 7 can be 10^3 to 10^4 higher. This fact can be utilized to quench or stop an exchange experiment so its extent can be measured. Since the structure of the I-A^k is known H/D exchange rate can provide a rapid method to determine the structure of I-A^k-peptide complex without crystallizing and also shed light on the solution dynamics of this complex.

In order to probe the I-A^k-HEL 48-62 complex peptide-amide-hydrogen exchange rate, a soluble form of the complex was made by oligonucleotide-directed cDNA mutagenesis with a covalently attached HEL 48-62. The structure of this complex has been recently resolved. After proteolysis the complex is occupied by a unique peptide. The exchange results were analyzed using LC-MS/MS, which unlike NMR is not limited to small proteins. The protocol begins with completely deutrating the MHC in D_2O. H/D back exchange is initiated by diluting the complex solution in H_2O at pH 7.4 at 39º C for varying times. The exchange is stooped by lowering the pH to 2.75 at 0º C. The peptide deuterium content and distribution is investigated by rapid LC separation and product ion spectra of the deutrated peptide. The exchange rate of each NH on the peptide is determined by fitting the data to a multiple first order rate kinetic model. A new data processing algorithm and a dedicated computer

program were developed to deconvolute the deuterium content from other isotopic contributions and calculate, from time-dependent product-ion spectra of the deuterium-enriched peptide, the rate constants for hydrogen exchange at most of the amide linkages of the bound peptide. The fitting method is fitting the rates to the data by starting from the smallest fragment and fitting one rate constant at a time for each consecutive fragment. The fit is accomplished by using the gradient free method of Rosenbrock[4]. The error function for the search is the root mean square of the difference between the experimental derived deuterium distribution and the model.

The amount of exchange is related to the extent of protection afforded the antigenic peptide in the MHC and is remarkably consistent with what is seen in the crystal structure.

References

1. Stern, *et al*., Nature, **368**, 215; Fremont *et al*., Science **272**, 1001.
2. Nelson, *et al*., Proc. Natl. Acad. Sci. USA 1997, **94**¸ 628; Parra-Lopez, *et al*.; J. Immunol., 1997, **158**, 2670; Gugasyan, *et al*.; J. Immunol., Submitted.
3. Liu, *et al*., J. Am.Soc. Mass Spectrom. 1994, **5**, 19-28
4. Rosenbrock, *et al*., Computer J. 1960, **3**, 175-184

Protection of Amide Bonds from H/D Back Exchange