

Structural Dynamics of MHC Class II HLA-DR Molecules: A Theoretical Perspective

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ABSTRACT: The enzymes that exhibit a domain movement, the conformational change induced by the binding of a substrate or coenzyme can be clearly characterized and therefore the opportunity exists to gain an understanding of the mechanisms involved. The domain movements in the immune system database of protein domain movements are those found by applying the DynDom program to pairs of crystallographic structures contained in Protein Data Bank files. For each pair of structures cross-checking ligands in their Protein Data Bank files for ligands that contact the enzyme in one conformation but not the other, the non-redundant database of protein domain movements was refined down to a set of MHC class II HLA-DR molecules where a domain movement is apparently triggered by the binding of a functional ligand. The presentation at a single website of data on interactions between a ligand and specific residues on the enzyme alongside data on the movement that these interactions induce, should lead to new insights into the mechanisms of these enzymes in particular, and help in trying to understand the general process of ligand-induced domain closure in enzymes.

KEYWORDS: MHC class II HLA-DR , enzyme, DynDom and Functional dynamics

I. INTRODUCTION

Enzymes are flexible molecules that change conformation upon ligand binding [1,2]. However, there is considerable variation in extent of that conformational change. A database study has shown that movements in enzymes upon substrate binding are generally small [3], and the extent of movement may depend on the actual reaction mechanism [4]. The complexity and variability of conformational change that enzymes exhibit upon ligand binding that makes their study so difficult. To overcome this, we report here on a database specifically devoted to enzymes with a domain movement upon ligand binding. Domain movements can be well characterized, defined, their relative movements can be described in terms of interdomain screw axes (hinge axes), and the hinge-bending regions can also be identified. This ability to characterise domain movements together with the fact that they are generally quite large (large rmsd between the two structures) also means it is possible to decouple them from uninteresting conformational differences that may be due to noise or reasons unrelated to the binding event. In enzymes with a domain movement the standard view is that the ligand binds to the open conformation and subsequently causes it to adopt a closed conformation where the ligand is surrounded by the enzyme in a highly specific environment. There are a number of different models of the kinetics of ligand binding and protein conformational change in domain proteins. The generation of short main-chain segments by use of a sliding window and the calculation of rotation vectors associated with the rotation of these segments between the two structures is the key function. By treating the components of these rotation vectors as coordinates in a "rotation space", segments that rotate together, perhaps comprising a rigid domain within the protein will have rotation points co-located. The domains identified as clusters of rotation points are identified using the k-means clustering method and are modelled as 3-dimensional normal distributions. Rotation points that have the dual property of lying outside the ellipsoids and in moving along the protein chain are from segments that connect the domains, are assigned "bending" rotation points. The residues associated with the bending rotation points are assigned as bending residues. Further details can be found in the DynDom1.50 paper [5]. In this

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study we have focussed on those cases where the domain movement is induced by the binding of a functional ligand to an enzyme MHC class II HLA-DR molecules [6].

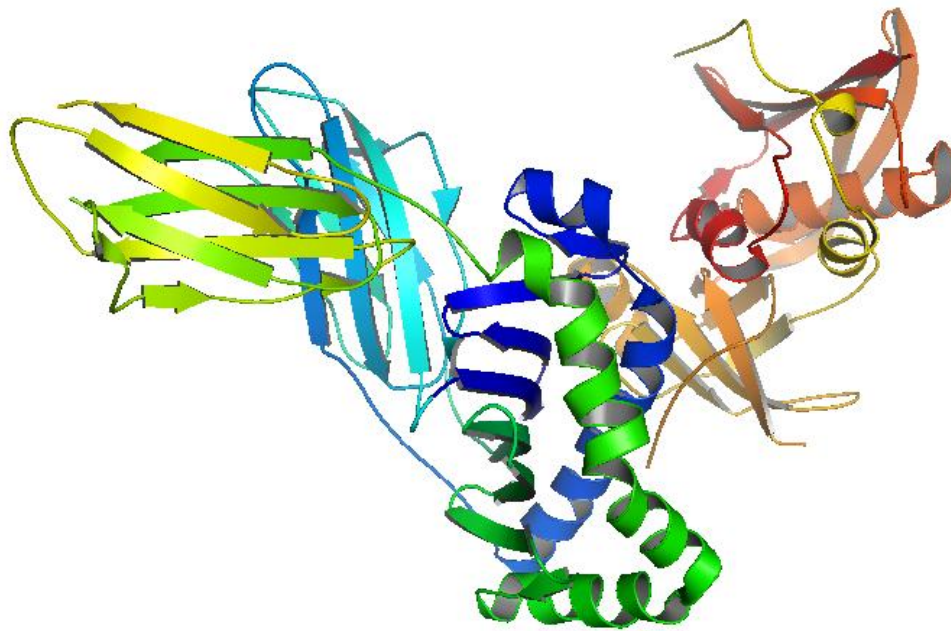


Figure 1: The schematic representation of HLA-DR class II MHC molecules

II. MATERIALS AND METHODS

Dataset Preparation

Here the methods used to extract domain movements caused by the binding of a functional ligand to an enzyme are described. This involved the selection of enzymes from the non-redundant database of protein domain movements, the selection of those enzymes where a ligand is present in at least one of the structures, the verification of the ligand as a functional ligand, and the final selection of those cases where the ligand could have triggered the conformational change upon binding. The current DynDom database [6] of protein domain motions provides a comprehensive and non-redundant dataset of protein domain movements based on the DynDom (version 1.50) methodology [5,7,8]. Each movement is defined by a pair of homologous protein chains in different conformations solved by X-ray crystallography. The database used here are protein families derived from the November 2012 release of the PDB. The website can be found at: <http://www.rcsb.org/pdb/home/home.do>. The domain movements in the protein are expressed by the dynamics of the existing chains and are being used for the DynDom analysis.

Functional Ligands

For each protein chain a list of all the compounds was compiled for its assigned EC number(s). This list (one for each chain) was termed the "KEGG-ligand list". For each protein chain, its PDB-ligand list was matched to its KEGG-ligand list by cross-checking for similar chemical formulae. If the difference in the number of heavy atoms between the two formulae were less than or equal to two, a match was assigned, meaning that the PDB ligand was considered to be a functional ligand

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(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 6, June 2015

for this protein chain. The trigger ligand is a contact ligand that is present in one conformation but not the other, i.e. it has caused the conformational change upon binding to the enzyme. It is the union of two non-overlapping sets.

III.RESULT AND DISCUSSION

Presentation at Website

The web-interface to the database is implemented using JAVA Server Pages (JSP) and servlets. The database software itself is PostgreSQL. The front page lists the enzymes giving their names, EC numbers, PDB accession codes and chain identifiers for each protein pair. The protein structure is coloured according to domain (blue or red) and interdomain bending regions (green).[9]

The "Run DynDom" form is used to identify the two conformations. Output from the DynDom program is shown in **Table 1**. It shows the number of residues used for the analysis and the backbone root mean-square deviation (RMSD) from a least-squares superposition of the two proteins with a cut-off value of RMSD larger than 0.1Å.

Table 1: The cluster of domains generated in DynDom

Dyndom Output
number of residues used for analysis: 179
rmsd of whole protein best fit: 0.80Å
number of clusters: 1
number of clusters: 2
found cluster for which all domains are less than minimum domain size
number of clusters: 3
found cluster for which all domains are less than minimum domain size
number of clusters: 4
number of domains equal to or larger than minimum domain size: 2
ratio of interdomain to intradomain displacement: 1.31
domain pair accepted
number of clusters: 5
found cluster for which all domains are less than minimum domain size
exiting clustering routine
rmsd of fixed domain best fit: 0.56Å

The above table shows the clustering process of DynDom. These clusters are used to identify the domain motions by a least squares fitting of the selected conformations given as the input. For each domain pair, the ratio of inter-domain to intra-domain displacement is given as output and should be greater than the minimum value (1.0). Such pairs would be accepted for the next stage of analysis, i.e. the determination of inter-domain screw axis and the bending regions. Detailed knowledge on the procedure adopted by the DynDom in describing the domain motions can be found in the work reported by Hayward and Lee [5].

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 6, June 2015

Table 2 shows the input parameters used for DynDom analysis. The minimum domain size and ratio that refers to the ratio of inter-domain to intra-domain displacement are fixed at 20 residues and 1.0, respectively, for all runs and are followed for the entire analysis. The window length is set initially at 5 residues.

Table 2: The Input parameters for DynDom analysis

Property	Value
Conformer 1 (1D5M)	B-chain of 1D5M
Conformer 2 (1D6E)	B-chain of 1D6E
Window Length	5
Minimum ratio	1.0
Minimum domain size	20

Table 3 shows the domains section, in which the segments forming the domain are given. The residue numbers are taken from conformer 1 and the numbering of conformer2 differs from conformer1. Each domain is given an identifying number. Domain number 1 comprises 94 residues and the backbone RMSD between the two conformations is 0.56Å. Domain 2 comprises 79 residues and has an RMSD of 0.37Å

Table 3: Identified Domains

Domain	Size	Backbone RMSD (Å)	Residues
1	94	0.56	86 - 188
2	79	0.37	7 - 85

Figure 2 shows a pairwise alignment of both sequences colored according to domain or bending region. The numbering is taken from their respective PDB files and the sequences themselves come from the ATOM record of the PDB file. The sequences between the two chains need not be identical but according to the construction of the non-redundant database, they will have a 90% or greater sequence identity with the representative of their family .[7]

Figure 2: The pairwise alignment of the sequences of conformer 1 and conformer 2.

1D5M_EDIT.PDB(B) : DTRPRFLEQVKHECHFFNGTERVRFLDRYFYHQEEYVRFSDVGEYRAVT	_____
:	_____
1D6E_edit.pdb(B) : *TRPRFLEQVKHECHFFNGTERVRFLDRYFYHQEEYVRFSDVGEYRAVT	_____
:	_____
1D5M_EDIT.PDB(B) : ELGRPDAEYWNSQKDLLEQKRAAVDTYCRHNYGVGESFTVQRRVYPEVTV	_____85_____
:	_____
1D6E_edit.pdb(B) : ELGRPDAEYWNSQKDLLEQKRAAVDTYCRHNYGVGESFTVQRRVYPEVTV	_____85_____
:	_____
1D5M_EDIT.PDB(B) : YPAnLLVCSVNGFYPGSIEVRWFRNGQEEKTGVVSTGLIQNGDWFQTLV	_____

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Vol. 4, Issue 6, June 2015

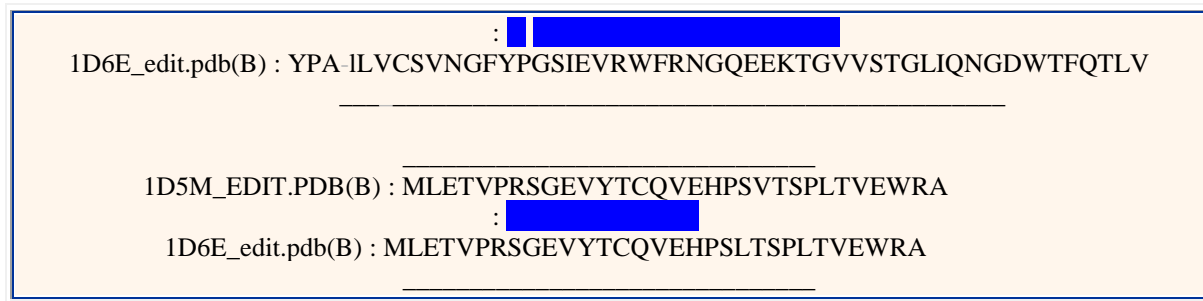


Figure 3 depicts the inter-domain motions. The domain 1 describes the rigid domain and the moving domain is described by domain 2. The residues 85 and 86 act as the hinge domain for the dynamics of domain 2 and is observed that this motion evolves with an angular rotation of 4.1 degree.[8,9]

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 4, Issue 6, June 2015

Figure 3: Domain Pairs

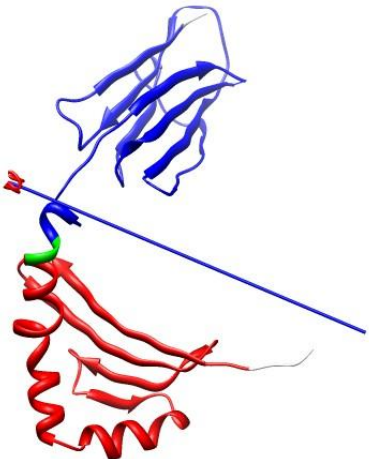
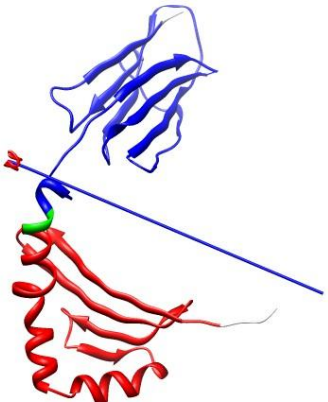
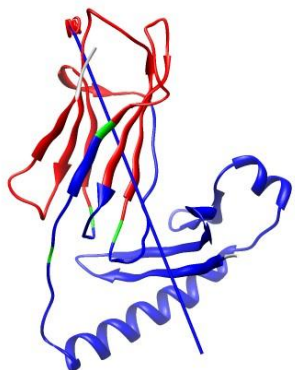
Property	Value	Domain motions
Fixed Domain (blue)	1	
Moving Domain (red)	2	
Rotation Angle (deg)	4.1	
Translation (A)	0.2	
Closure (%)	95.9	
Bending Residues (green)	85 - 86	

Figure 4 depicts the two conformations as well as an arrow which depicts the interdomain screw axis. The domain and bending regions of the first conformer will be colored according to the coloring format of Dyndom.[10-12]

Figure 4: View of domain motions with the help of screw axis

Chain B of 1D5M	Chain A of 1D5M
	

The information depicted in Table 4 is given for each bending segment. Table 4 shows the result for the bending region extending from residue 85-86. The first two columns of data show the distance of the alpha-carbon atom of residue *i* from the inter-domain screw axis for both conformations. The next two columns present the change in the psi-dihedral for residue *i*, and the change in the phi-dihedral of residue *i*+1, between the two conformations. The following columns give

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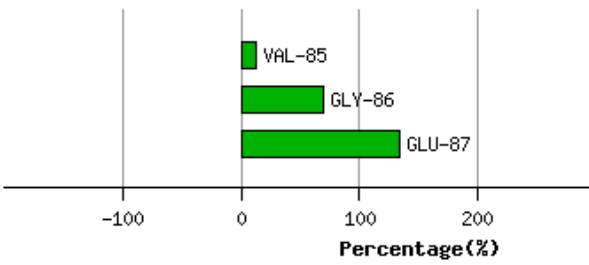
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Vol. 4, Issue 6, June 2015

the angle between the psi-dihedral axis and the inter-domain screw axis in both conformations. The final column is explained by first explaining the meaning of the plot. Bending regions are where the transition occurs between the two rotational properties of a pair of domains. The plot in Table 4 is meant to reveal to which domain the residue indicated is dynamically closest. In the plot 0% indicates that the residue is rotating with the domain from which it originates which in this case is domain 2, as residue 85 is part of that domain (Table 3). 100% indicates that the residue is rotating with the domain in which the bending residue segment terminates which is domain 1, as residue 86 belongs to domain 1 (Table 3). These percentages are calculated by evaluating the degree to which a residue is rotating with the rotating domain (2 in this case) and is determined by calculating the rotation of the tetrahedron formed from the atoms N,C-alpha,C-beta, and C and comparing it to the rotation of domain 1. In this case residue 85 has a value of 20%. Glu87 however has a value of about 140% which means it is rotating in the same direction as domain 2 relative to domain 1 but rotates a little more than the overall rotation of domain 1. In any case, this means that Glu87 is certainly closer associated with domain 1 than domain 2. The transition is seen to occur mainly between Gly86 and Glu87. The phi-dihedral of Glu87 changes by -4.5degrees compared to -2.7 degrees for Gly 86. The final column in Table 4 titled "Percentage Progress" gives the difference in the percentages of the corresponding two residues shown in the plot. The data presented in Table 4 can be used to identify residues that are classic hinge-bending residues. Often there is considerable noise in this data and for interdomain rotations less than 20 degrees it may not be very informative.

Table 4: Bending Residue Dihedral Analysis

Residue i	Residue i+1	Distance of hinge axis to residue i in conformer 1 (A)	Distance of hinge axis to residue i in conformer 2 (A)	Change in psi (i) (deg)	Change in phi (i+1) (deg)	Angle of psi(i) axis to hinge axis conformer 1 (deg)	Angle of psi(i) axis to hinge axis conformer 2 (deg)	Percentage Progress
GLY-84	VAL-85	10.3	10.0	7.1	-5.5	76.9	77.1	-50.3
VAL-85	GLY-86	8.6	8.5	6.3	-3.4	71.1	67.4	56.7
GLY-86	GLU-87	5.8	5.9	-2.7	-4.5	59.8	63.8	64.0



Graph shows rotational transition at bending residues and can be used to identify hinge bending residues.

International Journal of Innovative Research in Science, Engineering and Technology

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Vol. 4, Issue 6, June 2015

The above table lists the bending regions that interact with the ligand and the key residues involved in the domain motion in MHC class II HLA-DR molecules.

IV.CONCLUSION

This manuscript describes the ligand binding information in the context of dynamical information for enzymes that exhibit a domain movement upon ligand binding. This observation would be of particular use to experts interested in a particular enzyme. In general, this study promotes the understanding of how ligands induce conformational changes in enzymes, and also about the kinetic models of ligand-induced domain closure in regard to inhibition analysis.

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