The immune system
Biophysical Chemistry 1, Fall 2010

B-cells and T-cells
Catalytic antibodies
Reading assignment: Chap. 14
B-cell or humoral immunity uses a protein G fold

To be able to specifically recognize the vast number of molecules, the antibodies or immunoglobulins produced by B cells need to have an enormous variability. How is this achieved? To explain this, we first need to describe the organization of the antibodies. Antibodies are built of repeats of the same type of domain, a β-sandwich with two layers of antiparallel β-strands (Fig. 14.1).

### 14.1.1 The IgG Molecule

Antibodies are built of two types of polypeptide chains, heavy and light. The light chains are always composed of two domains, each of which contains about 110 amino acid residues. There are two main types of light chains, κ and λ. The heavy chains have at least four domains. In mammals, there are five different antibody classes with different functional properties and locations in the organism: IgA, IgD, IgE, IgG, and IgM. Other vertebrates have a more limited setup of antibodies. They all have different types of heavy chains and can form different oligomers. In plasma, the IgG molecule is the most common type of immunoglobulin. It has two heavy and two light chains with a total of 12 Ig-domains (Fig. 14.2). The amino terminal domains of a heavy chain and a light chain pair to form the antigen-binding domains. An IgG molecule has two identical sites at which it can bind antigens.

**FIGURE 14.1**

The immunoglobulin (Ig) fold. Many proteins in the immune system have this fold, as well as proteins involved in cell adhesion and the nervous system. Left: ribbon representations of the fold of a constant domain and a variable domain (PDB: 1AQK, heavy chain). Right: a simplified representation of the β-sandwich that constitutes the Ig fold. The constant domain has a four-stranded and a three-stranded antiparallel sheet, but in the variable domain there are two extra β-strands C' and C'' (darker blue). The red connections between some strands in the variable domain are the complementary-determining regions, CDR1, CDR2 and CDR3, consecutively along the polypeptide chain. These regions form the antigen-binding surface.

![Diagram showing the IgG molecule and the Ig fold](image-url)
IgG’s have a characteristic Y-shape.

The Ig-domains normally interact in a pairwise manner. They can be hetero-pairs as in the pairs of domains between the heavy and light chains in the Fab fragments or homo-pairs as in the Fc fragment (Figs. 14.2 and 14.3). There are also homodimers of light chains called Bence–Jones proteins produced in large quantities by certain types of cancer cells.

**FIGURE 14.2**
The IgG molecule is built of four multidomain chains, two heavy (blue domains) and two light chains (red domains). The heavy chains are composed of four domains while the light chains have two domains. The light blue and red domains are the variable domains where the antigen binding occurs. The darker domains are the constant domains. The heavy chains are linked to each other and the light chains are each linked to one of the heavy chains by disulfide bonds (yellow). If IgG is treated by a proteolytic enzyme such as papain, the heavy chains are cleaved between the first and second constant domains such that three fragments are generated. The fragment composed only of heavy chain constant domains is called Fc (as in “fragment crystalline”). When it was first produced, it crystallized spontaneously in the dialysis tube! The two identical fragments are called Fabs (fragment antigen-binding). The antigen (purple) binds between the two variable domains of each Fab fragment.

**FIGURE 14.3**
A detailed structure of IgG in two orientations. The heavy chain consists of the VH, CH1, CH2, and CH3 domains and the light chain of the VL and the CL domains. The Fab units are seen above and have very flexible links to the Fc unit below. The chains are connected by disulfide bonds. All cysteines that form disulfide bonds are shown and the ones that connect the chains are indicated by arrows. The Fc unit has carbohydrate modifications at the CH2 domain, drawn as ball-and-stick models (PDB: 1IGT).
IgG’s can be rather flexible

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Examples of fab’s binding to protein antigens

**FIGURE 14.4**
The binding of monoclonal antibodies to their antigens. Top: the variable domains of the Fab fragment bind a lysozyme molecule (PDB: 1VFB). Bottom: an Fab fragment binds the protein neuraminidase from the influenza virus (PDB: 1NCA). The hypervariable loops (CDRs) are shown in green.

**FIGURE 14.5**
Light-chain genetic rearrangement and expression. The different genetic segments can be combined in very many different ways. The undifferentiated cell has the complete set of antibody genes. During differentiation, a V gene becomes linked to a J gene in a random way. In the subsequent transcription of the DNA to a pre-mRNA, a further elimination is made. The mRNA is subsequently spliced into a mature mRNA that is translated into a specific light chain.

```
V1   V2   Vn-1  Vn  J1  J2  J3  J4  J5  C
V3   V4   J2   J3   J4   C
V4   J2   J3   J4   C
V4   J2   C
```

Gene elements for the light chain

Recombination

Transcription

Splicing

Translation
Genetic selection processes are key.

The Immune System

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Gene elements for the light chain

\[ V_1 \quad V_2 \quad V_{n-1} \quad V_n \quad J_1 \quad J_2 \quad J_3 \quad J_4 \quad J_5 \quad C \]

- Recombination
- Transcription
- Splicing
- Translation
The light chain, the β2-microglobulin subunit, is mainly associated with the α3 domain of the heavy chain. In class II, two chains both contribute elements to the peptide-binding site (Fig. 14.7). Nevertheless, the binding site for the peptide is designed in the same way in both types of MHC. The base is built from an eight-stranded β-sheet with a helix on each side of the peptide. The molecular organization resembles a hot dog.

MHC class I present peptides derived from intracellular degradation of proteins in the cytosol whereas class II present peptides from degradation of extracellular antigens in endosomal compartments.

The class I MHC molecules usually bind peptides 8–10 residues in length. The conformation of the peptide is extended with anchor residues bound in specificity pockets that differ in the alleles of MHC molecules. Since the ends of the binding site are closed, longer peptides will bulge when bound. In the class II binding site, the bound peptide adopts the conformation of a left-handed polyproline helix. The binding site is open at both ends allowing larger peptides to protrude at either end. Thus, MHC class II can bind longer peptides than class I.
Basic ideas of catalysis (again)
Catalytic antibodies

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Binding Energy and Catalysis: The Implications for Transition-State Analogs and Catalytic Antibodies

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Figure 1. Thermodynamic box illustrating relationship between ground-state and transition-state binding for an enzyme with a single substrate.
Testing the transition state stabilization idea

\[
\text{BnO}^-\text{N}^{\cdot}\text{O}^{\cdot}\text{P}^{\cdot}\text{O}^{\cdot}\text{R}^{\cdot}\text{Z}^{\cdot}\text{CO}_2^{-}
\]

\[
\approx
\]

\[
\text{BnO}^-\text{N}^{\cdot}\text{O}^{\cdot}\text{C}^{\cdot}\text{NH}^{\cdot}\text{R}^{\cdot}\text{Z}^{\cdot}\text{CO}_2^{-}
\]

\( R = \text{H, Me, } i-\text{Bu, Bn} \)

- **nn**: \( Y = Z = \text{NH} \)
- **on**: \( Y = O, Z = \text{NH} \)
- **cn**: \( Y = \text{CH}_2, Z = \text{NH} \)
- **no**: \( Y = \text{CH}_2, Z = O \)

**Figure 3.** Comparison of \( K_i \) values for phosphonate inhibitors of thermolysin with \( K_m/k_{cat} \) values for the corresponding substrates\(^{23,65}\). The diagonal lines correspond to slopes of 1.
Proto-typical transition-state analogues

Oxabicyclic diacid 9 that inhibits the chorismate mutases46 is much more compact than the expanded transition state 10 and does not emulate its charge separation.110 Clearly, we cannot expect even a faithful complement of an imperfect template to compete with an enzyme optimized to bind the true transition state.
T-cell immunity relies on peptide presentations.

There are also non-classical MHC molecules which bind glycolipids and lipopeptides to be presented to T cells. The variation in the binding sites on the different MHC molecules accommodates the wide range of peptides that needs to be presented (Fig. 14.8). The side chains of some of the residues in the bound peptide are exposed and are accessible for interaction with T-cell receptors.

**FIGURE 14.7** The binding of peptides to MHC class I (left) and class II (right) molecules. The peptide-binding site is a groove with a base of eight β strands and two α helices surrounding the peptide. The peptide is shown as a ball-and-stick figure. In MHC class I, some residues block the ends of the groove, while the ends of the groove are open in MHC class II.
Peptides bind to MHC in defined ways

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**FIGURE 14.7**  The binding of peptides to MHC class I (left) and class II (right) molecules. The peptide-binding site is a groove with a base of eight β strands and two α helices surrounding the peptide. The peptide is shown as a ball-and-stick figure. In MHC class I, some residues block the ends of the groove, while the ends of the groove are open in MHC class II.

**FIGURE 14.8**  The structures of the peptides bound to MHC class I (above) and class II (below). The β sheets of MHC have been aligned but are not shown. They are located below the peptides. The class I peptides are shown in different colors for different lengths: 8 (yellow), 9 (red) and 13 residues (green). The binding groove is closed at the ends in class I; therefore, peptides of lengths longer than eight residues will bulge. (Reprinted with permission from Rudolph MG et al. (2006) How TCRs bind MHCs, peptides and coreceptors. Annu Rev Immunol 24: 419–466. Copyright Annual Reviews.)
T-cell immunity involves a complex of many proteins.

T-cell receptors (TCRs) are located on the T-cell surfaces. Apart from a transmembrane region and a short cytoplasmic tail, they have the same general domain structure as antibody Fab fragments. They have constant and variable Ig-like domains and are composed of \( \alpha \) and \( \beta \), or \( \gamma \) and \( \delta \) chains. Both types are linked by disulfide bridges in a manner similar to the Ig molecules. While the \( \alpha \beta \) TCRs interact with antigenic peptides bound to MHCs, the \( \gamma \delta \) TCRs bind directly to pathogen-derived glycoproteins or non-classical MHC molecules.
T-cell immunity, again

Antibody Fabs, the regions of the TCRs that interact with MHC plus bound peptide are called complementarity-determining regions (CDRs). The CDRs interact with exposed sidechains of the bound peptide but also with the MHC $\alpha$ helices that embed the peptide (Fig. 14.9). The variable domain of the $\alpha$ chain ($V_\alpha$) is in contact with the N-terminal part of the antigen peptide whereas $V_\beta$ contacts the C-terminal region. This binding frequently leads to a diagonal orientation of the peptide relative to the receptor, but the variation is significant. The relative orientation of the receptor and the MHC complex could be important for T-cell signaling, but there is no full understanding of how this is transmitted into the cell.

FIGURE 14.10

The interaction between the D1 domain of CD4 (cyan) and the MHC class II complex. The bound peptide is shown as a ball-and-stick model (PDB: 1JL4). Below: schematic view of the interactions between the TCR co-receptors CD8 and CD4 with MHC class I and II, respectively. The co-receptors interact with the underside of the MHC molecules, opposite the peptide-binding site.
14.2.2.1 CD8 or CD4 assist the TCR in its interaction with MHC

TCRs are assisted in their interactions with MHC molecules by the co-receptors CD4 and CD8. TCR, CD4, and CD8 are all anchored in the T-cell membrane. A number of crystallographic structures describe these molecules and their interactions. CD8 is a heterodimer (the subunits are called $\alpha$ and $\beta$) where each monomer is composed of an Ig domain, a long linker and a transmembrane helix. CD4 is a monomeric protein composed of four Ig domains (D1–D4) of which D1 contacts MHC class II.

CD4 and CD8 interact with almost the same conserved regions, opposite the peptide binding side, on the underside of MHC class II and class I molecules, respectively (Fig. 14.10). CD4 is also the primary cellular contact at infections with HIV1. CD4 interacts with the viral spike protein gp120. This interaction involves the same surface of CD4 but is much stronger than the interaction with MHC class II.

14.2.2.2 CD3 accessory molecules signal the state of the TCR molecules

TCRs have a very small intracellular domain, insufficient for transfer of signals to the cellular machinery. Instead, TCRs are associated with three types of CD3 accessory molecules that contain domains involved in intracellular signaling. There are two types of heterodimers ($\gamma\epsilon$ and $\delta\epsilon$) of CD3, and these associate with the two chains of the TCR and a type of homodimer molecule ($\zeta\zeta$) into a complex of eight chains, each traversing the membrane. The extracellular domains of the CD3 $\gamma\epsilon$ and CD3 $\delta\epsilon$ heterodimers consist of side-by-side interacting Ig folds.

**FIGURE 14.11** Left: a schematic illustration of the interactions between TCRs and CD3s in the T cells. The CD3 $\gamma\epsilon$ and CD3 $\delta\epsilon$ are heterodimers that interact with TCR. Their location in the membrane defines their interactions and the intracellular signals transmitted. Right: the extracellular domains of the CD3-$\epsilon/\delta$ dimer associate with an approximate twofold axis that is vertical in this view. The $\epsilon/\gamma$ dimer is formed in the same way. The N-termini leads to the trans-membrane region (PDB: 1XIW).